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ERRATA AND AUTHORS' EMENDATIONS

Page 68, first table, last column, "0.0177" should read "0.0117."
Page 72, next to last line, "0.0352" should read "0.0252."
Page 74, fifth line, "celloid" should read "colloid."
Page 477, legends for figures 2 and 3, "Trichina" should read "Trichinella."
Page 482, fifth citation the title of the book ends with the word "Heidelberg." The words "Von Christ" which follow are part of the name of Fuchs and should read "Von Christ Jos. Fuchs und, etc."

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STUDIES IN INHERITANCE OF CERTAIN CHARACTERS OF CROSSES BETWEEN DAIRY AND BEEF BREEDS OF CATTLE [1]

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INTRODUCTION

An outstanding need of present-day genetics is the analysis of the hereditary variations in the economically important domestic animals. It is the purpose of this paper to present a preliminary analysis of the data from the first-generation crosses of the prominent dairy breeds of cattle on the beef breed Aberdeen-Angus (*22*, *42*).[2]

This work has been undertaken as a link in the chain of evidence necessary to the final solution of the problems which are connected with the inheritance of milk and butter-fat production. Considerable work has already been done in this laboratory in the analysis of the variation in these characters without immediate regard to the inheritance of such variation (*27–38*). These studies, it is believed, lay the foundation for the accurate analysis of such characters, for without a comprehensive understanding of the normal fluctuation of such quantitative character as milk production, it is practically impossible to determine such inheritance.

Further, the only method now known by which an adequate analysis of these laws of hereditary can be made is by hybridization experiments so carefully planned that the segregating factors may be analyzed separately. To that end a crossbred herd is being formed to accumulate as much material as possible for the analysis of such hereditary variation. This crossbred herd has now gone into its second generation. It seems wise, therefore, to make a preliminary analysis of the data of the first-generation herd.

This paper will have as its object the analysis of the inheritance of the more prominent characters of the first generation of this crossbred herd.

The plan of the work and its early prosecution was carried on by Dr. Raymond Pearl. Through the exigencies of the war the analyses of the data so far collected and the further prosecution of the work has fallen to the author. The results of this analysis are presented in the

[1] Reference is made by number (italic) to "Literature cited," p. 54–57.

[2] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 120.

following pages. The conclusions drawn from them are the author's, and he is alone responsible for them.

FOUNDATION HERD

The stock available for the crosses consisted of the following breeds in the relative proportions shown in Table I: Holstein-Friesian, Guernsey, Jersey, Ayrshire, and Aberdeen-Angus.

TABLE I.—*Breeds available as foundation stock for the crossbred herd*

Breed.	Male.	Female.
Holstein-Friesian	2	3
Holstein-Friesian grades	.....	37
Guernsey	2	16
Jerseys	1	10
Maine State Jerseys	.....	6
Maine State grades	.....	1
Ayrshire	1	8
Angus	2	5
Total, all breeds	9	86

It will be noted that there were a number of grades carrying much Holstein-Friesian blood. These were not used, since to carry convincing proof the ultimate results must be based on experiments with stock bred for some generations for the characters which were to be studied: milk, butter fat, and beef; otherwise the results might be explained by the heterogenous mixture of factors contained in the grades. The stock, together with the breeds which were finally used in the foundation crosses, is shown in Table II. They are all animals of good breeding and come from lines productive in milk, butter fat, or beef production, as the case may be.

It seems well at this point to consider the qualities of this foundation herd in respect to its inbreeding and possibilities of increased vigor due to heterosis of the crossbred progeny. Studies of the known inbreeding of this foundation herd have been made. The choice of a definite numerical measure of this inbreeding is necessary to any adequate study. Several such figures have been given to the students of inbreeding through studies of this laboratory (*27–38*). It is thought wise to use only one of these measures—namely, the coefficient of inbreeding—as this measures the total amount of inbreeding which has taken place in the pedigree of a given animal. Table III gives this amount of inbreeding in the successive generations, up to the fourth, known to have taken place in the pedigree of the animals used in this foundation herd. It will be noted that these inbreeding coefficients are, in general, low, compared with those of the animals already studied in this laboratory. This indicates that inbreeding would have little effect in increasing the vigor or production by the animals of the crossbred herd.

TABLE II.—*Parents of the crossbred herd*

Breed and animal.	Times bred.	Breed and animal.	Times bred.
JERSEY:		GUERNSEY:	
Lakeland's Poet (102603)	6	Lady Primrose's Governor of the Fountain (18328)	1
Columbia's Fox (126386)	1	Canada's Cresua (44386)	2
Flora's Golden Poetess (264927)	2	College Creusa (25661)	3
Rosalie (MSJHB 4887)	3	Creusa of Orono 3d (34228)	2
Ruth 8th (MSJHB 4457)	(a)	College Gem (40037)	1
Flying Fox's Flora (274051)	2	College Creusa 2nd (34227)	2
College Ruth (MSJHB 4895)	1	Creusa's Lady (53234)	1
Rue Victoria (273096)	2	College Gem 2nd (53235)	1
Columbia Brown Bessie (148551)	1	AYRSHIRE:	
Lassie of M. F. (297736)	1	Dot Alaska (29353)	3
HOLSTEIN-FRIESIAN:		Maple Grove Netta (29307)	2
Delva's University De Kol (146774)	1	Orono Netta (38832)	1
Johanna Lad Manor De Kol (41913)	1	ABERDEEN-ANGUS:	
Taurus Creamelle Hengerveld (98482)	16	Kayan (167617)	21
Pauline Posch (81048)	4	Eventime 4th (155526)	3
Delva Johanna De Kol (33910)	2	Hearthbloom (147141)	3
		Orono Madge (192781)	2
		Orono Ellen (192783)	2

(a) Twins once.

TABLE III.—*Amount of known inbreeding in the foundation herd*

Name of animal.	Known repeated ancestors' generations.				Total known repeated ancestors for four generations.	Coefficient of inbreeding.
	1	2	3	4		
Canada's Creusa (44386)	.....	.....	2	.....	4	25.000
College Creusa (25661)	.....	.....	2	.....	4	25.000
College Creusa 2nd (34227)	.....	.....	.....	2	2	12.500
College Gem (40037)	.....	.....	.....	.....	0	.000
Columbia's Brown Bessie (148551)	.....	.....	.....	1	1	6.250
Columbia's Fox (126386)	.....	.....	.....	.....	0	.000
Creusa's Lady (53234)	.....	.....	.....	.....	0	.000
Creusa of Orono 3rd (34228)	.....	.....	.....	2	2	12.500
Delva Johanna De Kol (146774)	.....	.....	.....	1	1	6.250
Delva's University De Kol (133910)	.....	1	.....	.....	4	25.000
Eventime 4th (155526)	.....	.....	.....	1	1	6.250
Kayan (167617)	.....	.....	.....	1	1	6.250
Hearthbloom (147141)	.....	.....	.....	.....	0	.000
Flora's Golden Poetess (264927)	.....	.....	1	1	3	18.750
Flying Fox's Flora (274051)	.....	.....	.....	.....	0	.000
Lady Primrose's Governor of the Fountain (18328)	.....	.....	.....	1	1	6.250
Lakeland's Poet (102603)	.....	.....	.....	3	3	18.750
Lassie of M. F. (297736)	.....	.....	1	.....	2	12.500
Pauline Posch (81048)	.....	.....	.....	.....	0	.000
Johanna Lad Manor De Kol (41913)	.....	.....	1	.....	2	12.500
Rosalie (MSJHB 4887)	.....	.....	.....	.....	0	.000
Rue Victoria (273096)	.....	.....	.....	2	2	12.500
Ruth 8th (MSJHB 4457)	.....	.....	.....	.....	0	.000
Taurus Creamelle Hengerveld (98482)	.....	.....	1	1	3	18.750
Maple Grove Netta (29307)	.....	.....	.....	.....	0	.000
Average inbreeding	.....	.....	.....	.....	.....	8.654

a All animals that can not be traced fairly completely for the four generations are excluded from this table.

GENERAL PLAN OF THE MATINGS

The experiments for the study of the inherited characters of the different breeds were intended to include those breeds where careful selection of these characters had been made. Before passing to a study of these characters in the F_1 hybrids it seems necessary to have clearly in mind the characters of the breeds used as parents so that a definite conception of the breed and type differences may be had. Toward this end Table IV has been drawn up in the simplest form possible.

TABLE IV.—*Contrasting characters of the parental breeds of the crossbred herd*

Character.	Jersey.	Guernsey.	Ayrshire.	Holstein-Friesian.	Aberdeen-Angus.
Body color	Fawn or dun	Light fawn or dun.	Red	Black	Black.
White markings.	Often absent	Present	Present	Present	Often absent.
Switch color	Black or white.	Light fawn or white.	Red or white	Black or white.	Black.
Muzzle pigment.	Black	White	Black	...do	Do.
Tongue pigment.	...do	...do	...do	...do	Do.
Horns	Horns	Horns	Horns	Horns	Polled.
Conformation	Dairy	Dairy	Dairy	Dairy	Beef.
Milk quantity	Medium	Medium	Medium	Large	Low.
Milk quality	High	High	...do	Low	High.

By comparing the various characters which the different breeds exhibit, as seen in Table IV, the following character differences are brought out. The coat variations offer a range of color from black to almost white. This white is divided into more or less definitely centralized white areas, each one of which presumably behaves distinctly. The tongue colors of the Jersey and Guernsey are such that colors from white to black are available. The typical conformation of each breed is distinct, offering many points of difference. Further differences which seem rather too obvious to describe are horns, secretions, skin texture, general body build, mammary development, temperament, and the physical and chemical character of the milk. In all it is believed that the choice of the foundation stock has been almost ideal, for in each breed chosen there has been years of selection for some of the above-mentioned characters whose inheritance is to be studied.

In this connection it may be objected that the inheritance of these characters are not economically important. This is in a sense true; yet the intimate association which exists between the hereditary units necessitates the studying such things as coat color and tongue color for the full analyses of the economically important problems. It is the purpose of this preliminary paper on the first generation hybrids to lay the foundation for acquiring exact knowledge of such things as milk and beef production.

CROSSBRED HERD

TIME OF BEGINNING MATINGS

The crosses herein described are the result of matings since the spring of 1913. For the number of animals there were to work with this is satisfactory progress, and it is expected that with reasonable success the first-generation crosses will soon be complete.

PLAN OF MATINGS

The plan calls for reciprocal matings of all of the important dairy and beef characters. At first there were some crosses made which were later found to be hampered by the fact that it was impossible to keep the bulls necessary for the F_2 generation. Consequently only the heifers were saved for future breeding purposes. The breeds chosen for the foundation of the F_1 Mendelian herd were Jersey, Holstein-Friesian, and Aberdeen-Angus. The ultimate choice of these rests on the fact that these breeds have all the outstanding characters which were desired for analyses, such as low and high milk production, low and high percentage of fat, poor and good beef qualities. Besides these, the minor characters of color, secretions, and conformation offer considerable range of variability for analyses in conjunction with the analyses of the economic characters. In handling the Mendelian herd and its parential generation, it is the plan to have them under as nearly the same conditions as the rest of the herd animals as it is possible. They are raised side by side with the other herd animals and treated in exactly the same way, by the same herdsman. In the breeding of the animals a rack is used wherever there is a marked difference in size or where it is desirable to facilitate the matings. After the mating has taken place, the herdsman fills out one of the service record blanks shown in figure 1.

If the first service fails, another service is given at the next period of heat. Each of these service blanks are filled out and carefully filed. When a birth takes place, the herdsman files another blank recording such birth. The corresponding service and birth blanks are then filed together. Each animal is tagged at birth with a number corresponding with that on its birth blank. These tags are the common metal tags put through the cartilage of the ear. Besides this, when the animals are old enough, they are branded on the shoulder with their distinguishing number. Any chance in mistake of pedigree of these animals is thus reduced to a minimum.

All of the animals are kept until they are 200 days old, when they are measured for the study of conformation in relation to milk production. Besides this description, the animals are carefully examined and a description of the color and other outstanding features made. Photographs of all of the animals are taken before they are sold and kept in the permanent files of the Biological Laboratory for reference.

DESCRIPTION OF MATINGS

Before passing to the analyses of the individual F_1 hybrids it is well to have clearly what each individual mating was and what differences between them could be expected. To this end Table V has been drawn up to show the matings which produced each crossbred.

FIG. 1.—SERVICE AND BIRTH RECORDS USED AT MAINE EXPERIMENT STATION.

Service record blanks are filed numerically with the herdsman. When a service is made, the service record blank is filled out immediately and filed by the author. The resulting birth is described on the birth record. The corresponding service and birth records are connected in the office.

MAINE AGRICULTURAL EXPERIMENT STATION, ORONO, MAINE

THIS INFORMATION WILL BE HELD STRICTLY CONFIDENTIAL

This blank should be filled out immediately after the service is completed, and mailed in an addressed envelope furnished you, to the Agricultural Experiment Station, Orono, Maine.

SERVICE RECORD

Date. Hour of service This record made by

BULL used. (Name.) Breed.

Is the bull registered? If so, give reg. No.

Owner of bull—Name. Address.

Age of bull. No. of coverings at this service.

COW served. (Name.) Breed.

Is this cow registered? If so, give reg. No.

Owner of cow—Name. Address.

Age of cow. When did she calve last? (Give month, day and year.)

How many times has she been in heat since calving INCLUDING this heat?

GIVE THE HOUR (AND DAY) WHEN IT WAS FIRST NOTICED THAT THE COW WAS IN HEAT BEFORE SHE WAS PUT TO THE BULL THIS TIME

How many hours had the cow been in heat before she was served?

(Do not write in this space.)

SERIES. NUMBER.

MAINE AGRICULTURAL EXPERIMENT STATION, ORONO, MAINE

THIS INFORMATION WILL BE HELD STRICTLY CONFIDENTIAL.

One of these blanks should be filled out immediately after each calf is born, EVEN IF IT IS A PREMATURE BIRTH (ABORTION). Mail to the Agricultural Experiment Station, Orono, Maine.

BREEDER'S BIRTH RECORD

Date of birth. Hour of birth. Record made by.

Was the calf male or female? (Make especial note of twin births.)

Weight of calf at birth.

Sire of calf. (Give name and breed.) Reg. No.

Dam of calf. (Give name and breed.) Reg. No.

How long was the dam dry before calving?

At what hour (and day) did labor begin?

Has the dam ever aborted? (If so give particulars)

Note any peculiarity about the birth or the calf which interests you and might interest others, especially abortions and monstrosities.

(Do not write in this space.)

Sex entered.....

Duration of gestation days hrs. (20 x)+

SERIES. NUMBER.

TABLE V.—*Number, sex, and parentage of animals of the crossbred herd*

Calf No.	Sex	Dropped.	Name of sire and registry No.	Breed of sire.	Name of dam and registry No.	Breed of dam.
		1914.				
0..	M.	Mar. 28	Lakeland's Poet (102603).	Jersey.....	Delva Johanna De Kol (146774).	Holstein-Friesian.
1..	F.	Apr. 5	.....do.....	.....do.....	Pualine Posch (81048).	Do.
2..	F.	Nov. 22	Delva's University De Kol (133910).	Holstein-Friesian..	Canada's Creusa (44386).	Guernsey.
3..	M.	Dec. 10	Johanna Lad Manor De Kol (41913).	.....do.....	Flora's Golden Poetess (264927).	Jersey.
		1915.				
4..	M.	Jan. 20	Taurus Creamelle Hengerveld (98482).	.....do.....	Rosalie (4887).....	Do.
5..	M.	Jan. 24	Kayan (167617).....	Aberdeen-Angus..	Dot Alaska (29353)..	Ayrshire.
6..	M.	Feb. 8	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Maple Grove Netta (29307.)	Do.
7..	M.	Feb. 13	Kayan (167617).....	Aberdeen-Angus..	Ruth 8th (4457)	Jersey (MSJHB).
8..	M.	Mar. 23	.....do.....	.....do.....	College Creusa (25661).	Guernsey.
9..	M.	Mar. 26	.....do.....	.....do.....	Pauline Posch (81048).	Holstein-Friesian.
10..	M.	Apr. 7	.....do.....	.....do.....	Creusa of Orono 3d (34228).	Guernsey.
11..	F.	Apr. 21	Lakeland's Poet (102603).	Jersey.....	Delva Johanna De Kol (146774).	Holstein-Friesian.
12..	F.	Apr. 22	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	College Gem (40037)	Guernsey.
13..	M.	May 4	Columbia's Fox (126386).	Jersey.....	Eventime 4th (155526).	Aberdeen-Angus.
14..	M.	June 6	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Flying Fox's Flora (274051).	Jersey.
15..	F.	Oct. 23	Lakeland's Poet (102603).	Jersey.....	Hearthbloom (147141).	Aberdeen-Angus.
16..	F.	Oct. 27	Kayan (167617).....	Aberdeen-Angus..	College Ruth (4895).	Jersey (MSJHB).
17..	M.	Nov. 8	.....do.....	.....do.....	Rue Victoria (273096)	Jersey.
		1916.				
18..	M.	Jan. 1	.....do.....	.....do.....	Ruth 8th (4457)	Jersey (MSJHB).
19..	F.	Jan. 1	.....do.....	.....do.....	.....do.....	Do.
20..	M.	Jan. 10	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Maple Grove Netta (29307).	Ayrshire.
21..	M.	Jan. 14	Kayan (167617).....	Aberdeen-Angus..	Dot Alaska (29353)..	Do.
22..	F.	Feb. 22	.....do.....	.....do.....	College Creusa (25661).	Guernsey.
23..	M.	Mar. 9	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Columbia Brown Bessie (148551).	Jersey.
24..	M.	Mar. 20	Kayan (167617)	Aberdeen-Angus..	College Creusa 2d (34227).	Guernsey.
25..	F.	Apr. 10	F_1 Crossbred (0).....	Jersey × Holstein.	F_1 Crossbred (1)....	Jersey×Holstein.
26..	F.	May 5	Kayan (167617).....	Aberdeen-Angus..	Creusa of Orono 3d (34228).	Guernsey.
27..	F.	May 25	Lakeland's Poet (102603).	Jersey.....	Orono Madge (192781).	Aberdeen-Angus.
28..	M.	June 17	Kayan (167617).....	Aberdeen-Angus...	Pauline Posch (81048).	Holstein-Friesian.
29..	F.	July 19	.....do.....	.....do.....	Creusa's Lady (53234)	Guernsey.
30..	M.	Aug. 29	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Orono Ellen (192783).	Aberdeen-Angus.
31..	M.	Sept. 11	Lakeland's Poet (102603).	Jersey.....	Eventime 4th (155526).	Do.
32..	M.	Sept. 25	Kayan (167617).....	Aberdeen-Angus..	Canada's Creusa (44386).	Guernsey.
33..	F.	Oct. 9	Lady Primrose's Governor of the Fountain (18328).	Guernsey.....	Hearthbloom (147141).	Aberdeen-Angus.
34..	F.	Oct. 17	F_1 Crossbred (0).....	Jersey×Holstein..	Rosalie (4887).....	Jersey (MSJHB).
35..	M.	Nov. 10	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian .	Flying Fox's Flora (274051).	Jersey.
36..	M.	Dec. 15	Kayan (167617)	Aberdeen-Angus..	Orono Netta (38832).	Ayrshire.
		1917.				
37..	F.	Jan. 5	.....do.....	.....do.....	Dot Alaska (29353)..	Do.
38..	M.	Jan. 13	F_1 Crossbred (10).....	Angus×Guernsey.	College Creusa (25661).	Guernsey.
39..	M.	Jan. 28	Kayan (167617).....	Aberdeen-Angus..	Rue Victoria (273096).	Jersey.
40..	M.	Feb. 9	.....do.....	.....do.....	College Creusa 2nd (34227).	Guernsey.
41..	F.	Feb. 9	.....do.....	.....do.....	College Gem 2nd (53235).	Do.
42..	F.	Mar. 25	Crossbred 0.....	Jersey - Holstein Friesian.	Flora's Golden Poetess (264927).	Jersey.
43..	M.	Apr. 30	.....do.....	.....do.....	Crossbred 1.....	Jersey-Holstein-Friesian.

TABLE V.—*Number, sex, and parentage of animals of the crossbred herd*—Continued.

Calf No.	Sex	Dropped.	Namd of sire and registry No.	Breed of sire.	Name of dam and registry No.	Breed of dam.
		1917.				
44..	F.	May 4	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian.	Orono Madge (192781).	Aberdeen-Angus.
45..	F.	May 13	Kayan (167017).....	Aberdeen-Angus..	Pauline Posch (81048).	Holstein-Friesian.
46..	F.	June 6	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian.	Lassie of M. F. (297736).	Jersey.
47..	F.	Aug. 6	.....do.....	.....do.....	Hearthbloom (147141).	Aberdeen-Angus.
48..	M.	Aug. 10	Crossbred 0.....	Jersey - Holstein-Friesian.	Crossbred 11.....	Jersey-Holstein-Friesian.
49..	F.	Aug. 15	Taurus Creamelle Hengerveld (98482).	Holstein-Friesian.	Crossbred 2.....	Holstein - Friesian-Guernsey.
50..	M.	Aug. 22	.....do.....	.....do.....	Eventime 4th (155526).	Aberdeen-Angus.
51..	M.	Aug. 27	.....do.....	.....do.....	Crossbred 12.....	Holstein - Friesian-Guernsey.
52..	F.	Oct. 11	.....do.....	.....do.....	Orono Ellen (192783).	Aberdeen-Angus.
53..	M.	Oct. 21	.....do.....	.....do.....	Rosalie (4887).....	Jersey (MSJHB).

An examination of Table V shows that at the beginning of the experiment there was a large preponderence of males. This is shown graphically in figure 2.

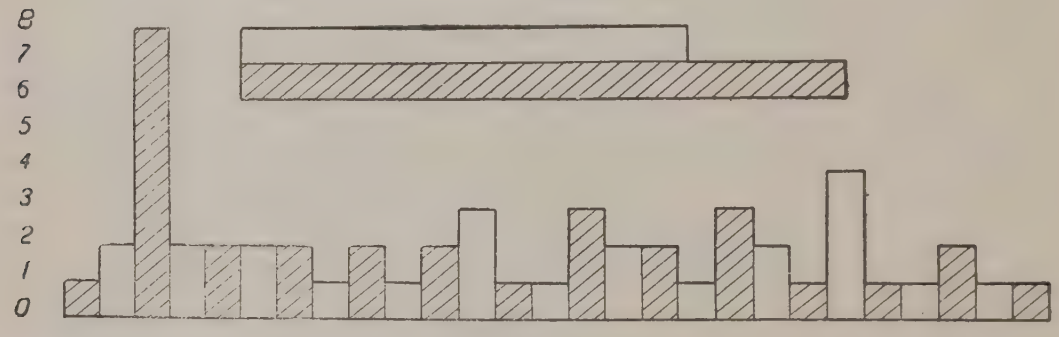

FIG. 2.—Diagram showing grapnically the number and sex sequence of the births composing the crossbred herd. The clear rectangles are female. The cross-hatched rectangles are the male.

This preponderance of males is shown by figure 2 to be due to a large excess of males in the first matings. In view of the fact that there is probably no relation between the time of service and the sex of the animals produced, this excess is to be explained on the basis of chance. Unfortunately, this first excess of males in the birth has not been completely neutralized, but still leaves a good number of males over the expected half. In the first 54 births there were 31 males to 23 females. Since the segregation of the sex-determining elements probably takes place in the male, it is interesting to see what is the relation between the individual bulls in regard to the sex of the offspring produced. The numbers as yet are rather too small to draw any definite conclusion, but are still large enough to be of interest. Table VI gives such a comparison of breeds.

TABLE VI.—*Relation of the sex produced to breed of bull*

Breed and name.	Male.	Female.
JERSEY:		
Lakeland's Poet	2	4
Columbia Fox	1	.....
Total	3	4
HOLSTEIN:		
Delva's University De Kol	.....	1
Johanna Lad Manor De Kol	1	.....
Taurus Creamelle Hengerveld	10	6
Total	11	7
ABERDEEN-ANGUS:		
Kayan	15	8
Total	15	8
GUERNSEY:		
Lady Primrose's Governor of the Fountain	.....	1
Total	.....	1
CROSSBRED:		
No	2	3
Total	2	3
Grand total	31	23

Table VI shows that the large number of males is principally due to two animals—Taurus Creamelle Hengerveld and Kayan. This increased number over half does not belong to any breed difference, but is probably simply a function of chance sorting.

DESCRIPTION OF THE PARENTAL HERD

The description of the parents and their crossbred offspring will be arranged in the following form in order to facilitate comparison. The description of the individual parents will be made first.

The description of the animals will be given in the following form: First, the sex, then the color and markings of the various parts of the body—muzzle, tongue, switch, horns, hoofs, and secretions—and then the general type that the offspring exhibits in regard to its breed characteristics. The further points considered will be in the following succession: Type as judged by dairy or beef production, number and placement of teats or rudimentaries, description of milk veins and wells, and lastly the capacity and quality of the udder.

The individual descriptions of the crossbred animals follow that of their parents. The mating is given first, then the number of the resulting offspring. The rest of the description of these crossbreds follows the same form as that of their parents.

DESCRIPTIONS OF ANIMALS IN PARENTAL HERD

Aberdeen-Angus Herd

Male: KAYAN (167617).—Solid black except for a small amount of white around rudimentaries. Muzzle black; tongue slate; switch black, with a few gray hairs scattered through it. No horns nor traces of them. Hoofs black, and secretions dark brown. The head is a good typical Aberdeen-Angus head. Well-rounded low-set bull, kept in rather low flesh for breeding purposes. Four rudimentaries; milk veins fairly large and long.

Female: EVENTIME 4TH (155526).—Solid black; muzzle black; tongue slate; switch black, with a few gray hairs in it. No trace of horns present. Hoofs black; secretions dark brown. The face is typically Angus. The shoulders, barrel, rump, fore and hind quarters are not deeply fleshed and lack the filling typical of Angus. In short, the cow has some traces of the dairy type; at the same time she has the deep, blocky body characteristic of the beef breed. Four fair teats and two rudimentaries. Fair-sized milk veins and wells. Udder large for such a low-milking breed (Pl. 1, A).

Female: HEARTHBLOOM (147141).—Solid black except for a few white hairs between the fore teats. Muzzle black; tongue black. Switch black, with a few gray hairs in it. No trace of horns or scurs. Hoofs black; secretions dark brown. Typical Aberdeen-Angus in shoulders, head, barrel, and fore and hind quarters of an animal in breeding condition. Four well-placed teats, udder of small capacity. Milk veins and wells small. A typical beef cow having none of the dairy points developed to any extent (Pl. 1, B).

Female: ORONO MADGE (192781).—Black except for a small white area on the teats. Muzzle and tongue black; switch black, with a few gray hairs in it. Horns entirely absent. Hoofs black; secretions dark brown. Face rather lacking somewhat in the Aberdeen-Angus type. It is rather too thin. Body lacks filling in chine and over the withers. Four teats and one rudimentary. Milk veins and wells are small. Udder of low capacity.

Female: ORONO ELLEN (192783).—Solid black except for a few white hairs on hind quarters of udder. Muzzle black; tongue black; switch black. No trace of horns; hoofs black. Secretions dark brown. A typical Aberdeen-Angus cow in low flesh. Four teats and one rudimentary on right side between the fore and hind teats. Udder of low capacity and meaty in texture.

Ayrshire Herd

Female: DOT ALASKA (29353).—Red, white, and black; black confined to outside of nostrils. Majority of the body is dark red. Spotting irregular in area, occurring on the shoulders, brisket, belly, flank, rump, and base of tail. Practically all of the white areas are flecked with the red islands. Muzzle black; tongue white; switch red and black, mixed with some gray. Horns are not at all "typy" of the Ayrshire, as they are too thin and not thrown upward sufficiently. Hoofs black; secretions orange. Face has too much dish and the body is too short and too low to the ground for the good type of Ayrshire animal. Udder rather small and only of fair shape. Milk veins and wells of medium length and size. The animal is much too small and light, even for the New England type, the type she most resembles.

Female: ORONO NETTA (38832).—Red and white; large broad star. Shoulders and brisket and front part of fore and hind legs white. The barrel and rump are white-spotted, each spot containing large irregular islands. Muzzle black; tongue black. White, heavy horns, pretty well up. Hoofs black; secretions small in amount and yellow. Dark-red nose, with a few gray hairs scattered through it. Rump not quite as level as would be expected of an animal typical of the breed. Hind quarters somewhat heavy. Udder small, especially in the fore quarters. Milk veins and wells small. Four well-placed teats (Pl. 1, C).

FEMALE: MAPLE GROVE NETTA (29307).—Color red and white. Much resembling the spotting of her daughter, Orono Netta. Muzzle black; horns heavy; black-tipped and thrown forward and up, as would be characteristic of the Ayrshire. Udder good-sized and well shaped. Well carried out in fore quarters. General type is that of the New England Ayrshire.

GUERNSEY HERD

MALE: LADY PRIMROSE'S GOVERNOR OF THE FOUNTAIN (18328).—Color a light yellow and white. White confined chiefly to the shoulders, legs, and belly. Muzzle is flesh-colored. Switch white. Horns coming out at right angles to head and curving slightly forward. Good Guernsey form both in head and in fore and hind quarters. Four well-developed rudimentaries (Pl. 2, A).

FEMALE: COLLEGE GEM (40037).—Color orange and white. White on belly, fore and hind legs chiefly. Tongue white; few white hairs on face; muzzle smutty; switch white. Horns large, thrown forward, up and back, black tipped. Hoofs white; secretions orange. General appearance is heavy for a dairy cow. Face a little short; body short; withers thick, well-developed; broad girth; rump fairly short; tail set high; twist well-filled. Udder small and poorly shaped. Veins and wells fairly large.

FEMALE: COLLEGE GEM 2ND (53235).—Color: Orange and white. White star on forehead. Two white spots on left shoulder. Belly white; short white stocking on left foreleg, extending as long white area along front of leg to brisket.

Hind legs are white below the knee. Muzzle white; tongue white; switch white. Horns long and slender, thrown up and back. Hoofs white; secretions orange, large in amount. The animal exhibits a fair Guernsey type. although rather light in body weight and heavy in shoulders. Udder of fair shape; teats rather well placed; medium-sized milk veins and wells.

FEMALE: COLLEGE CREUSA (25661).—Color orange and white, white star on forehead. Muzzle has a grayish area around it. Broad band of white extending across the shoulder and connecting with the white belly. Forelegs, brisket, and udder white. Broad band of white across the rump including the exterior end of the chime and running down on forelegs as far as the hoof. Muzzle white, with a few black spots on it. Tongue white; switch white. Horns heavy, thrown up and back. Hoofs white; secretions orange. The general type is that of a fair Guernsey. Udder is good size, although rather poor in shape. Milk veins and wells good size.

FEMALE: COLLEGE CREUSA 2ND (34227).—Color orange and white. Large white star. Udder white, broad band extending over rump to fore sides of white hindlegs; hindlegs are clear white to knee. Muzzle white; tongue white; switch white. Horns long, thin, thrown upward and curved forward and in. Hoofs white; secretions orange, large in amount. Nose narrow, strong, good dish and veins. General type is that of a fair Guernsey. Udder rather small, pendant, and poorly shaped. Medium-sized veins and wells.

FEMALE: CREUSA'S LADY (53234).—Color yellow and white. Star, white spot on both shoulders, extending over withers. Belly white; forelegs white on the inside; short stockings. White spot on left side of barrel. Rump and tail set white. Irregular white lines in front of hindlegs, extending down into clear white area below the knee. Muzzle, tongue, and switch white. Horns thrown forward and up. Hoofs white; secretions orange. Animal is rather light in weight and withers are a trifle heavy for a typical Guernsey animal. Milk veins lead to rather good wells (Pl. 2, B).

HOLSTEIN-FRIESIAN HERD

MALE: JOHANNA LAD MANOR DE KOL (41913).—Color black and white. Star. Broad band of white just back of the shoulders, extending around the body. Forelegs white, tail set white, hindlegs white, the white extending as irregular area upwards to the flank. Muzzle black, switch white. Horns large and thick, coming

out at right angles from head. Good Holstein-Friesian type; if anything the head has too much of the aquiline and perhaps might be considered chunky. Neck and shoulders are heavy. Crest is large. Rump well rounded. Throughout, the bull gives the appearance of a strong, masculine development.

MALE: TAURUS CREAMELLE HENGEVELD (98482).—Color black and white. White on face, shoulders, and belly. Stockings on fore and hind feet. Muzzle black; tongue and switch white. Large straight horns come out at right angles from head. Hoofs white, with black streaks in front; secretions dark brown. Four well-placed rudimentaries. Veins long, leading to good-sized wells. In the white areas the skin may be seen to be spotted with black. All in all the bull is a good large type of Holstein-Friesian (Pl. 2, C).

FEMALE: PAULINE POSCH (81048).—Color black and white. Broad white blaze. Throat white. Forelegs white. Broad band of white on both shoulders and over withers. Brisket and belly white. Irregular white area running from white hindleg over the flank and across white rump to connect on the other side with a white area coming from the other hindleg. Muzzle black and white. Tongue and switch white. Horns fairly heavy, curving forward and down. Hoofs black-and-white streaked; secretions dark brown. Good Holstein-Friesian type. Udder somewhat too pendant and lacks filling in the fore quarters. Milk veins tortuous and of good size. Wells good size.

FEMALE: DELVA JOHANNA DE KOL (146774).—Color black and white. White star. Brisket and dewlap white. White forelegs. Belly and udder white. Hindlegs white, the white areas extending irregularly onto the flank. Muzzle black. Tongue and switch white. Horns fairly heavy, turning forward. Hoofs white; have a black streak in front part; secretions dark brown. Udder has a tendency to be somewhat pendant. Veins large and tortuous. Fair-sized wells. The skin under the white areas can be seen to be black.

JERSEY HERD

MALE: LAKELAND'S POET (102603).—Color black, brown, and dark fawn. Head and shoulders dark brown to black, gray at base of horns. Back a dark fawn. Legs dark fawn. Hair extremely heavy on face. Muzzle black; tongue black, with a small white spot on tip; switch black. Strong horns turned forward and in. Hoofs black; secretions yellow. Rump a trifle long. Dewlap rather prominent. Four rudimentaries fairly well placed. Small milk veins and wells (Pl. 3, A).

MALE: COLUMBIA'S FOX (126386).—Color solid except for a small white spot on right stifle. Tongue black; switch black.

FEMALE: LASSIE OF M. F. (297736).—Color gray-fawn. Black spot on forelegs just above hoofs. Hindlegs light gray-fawn. Muzzle and tongue black. Switch black, with a few gray hairs in it. Horns turned forward and in. Hoofs black; secretions yellow. Face a little long; nose a little narrow. Withers somewhat heavy and a little too prominent for a perfect Jersey type. Udder of excellent proportions and of good size. Teats well placed; milk veins on right side branched, on the left side single (Pl. 3, B).

FEMALE: ROSALIE MSJHB (4887).—Color a light cream and black. Black on front side of face, forelegs, and hind quarters. Muzzle black; tongue black; switch black. Horns black and white, rather light, thrown forward and up; hoofs black; secretions yellow. The animal is not at all a good Jersey type because of the heavy withers, pronounced dewlap and barrel showing little tendency to the typical V-shape. Udder is rather small; teats small; milk vein on right side long, left side short and small.

FEMALE: RUTH 8TH MSJHB (4457).—Color solid orange. Muzzle black; tongue black; switch black. Horns fine, white tips. Hoofs black; secretions yellow. Face is overrefined. Dewlap and brisket too much in evidence. Vertebra wide apart.

Barrel medium-sized. Long level rump; fine clean bones. Cow in extremely poor condition. Udder rather good size, pendant; teats large and long; milk veins short and of fair size.

FEMALE: COLLEGE RUTH MSJHB (4895).—Solid color, yellow fawn, varying in shades. Dark on face, neck, and back. Light on nose, belly, and legs. Muzzle black; tongue white, background covered with small black spots; switch black. Horn slendor, turning forward and up, white base, black-tipped. Hoofs black; secretions yellow. Udder small, deeply cut between quarters. Small teats; good-sized veins, rather long and leading to good-sized wells.

FEMALE: RUE VICTORIA (273096).—Color solid, a general light cream. Muzzle black; tongue black; switch black. Horns heavy, turning forward and in. Hoofs black; secretions yellow. Face and neck are fair type. Shoulders heavy; brisket of good size. Barrel short; rump long and level. Udder rather small; teats well placed; milk veins good-sized and tortuous.

FEMALE: FLYING FOX'S FLORA (274051).—Solid color. Muzzle black; tongue black; switch black. Horns black-tipped, turn forward and in. Secretions yellow. Udder good size with well-balanced quarters. Teats well placed.

FEMALE: COLUMBIA'S BROWN BESSIE (148551).—Solid color. Muzzle black; tongue black; switch black. Horns turn forward and down. Udder pendent, hind quarters good; fore quarters rather poor. This may not be the natural condition, as the animal is quite old.

FEMALE: FLORA'S GOLDEN POETESS (264927).—Solid color, dark gray-fawn. Face and front part of forelegs dark. Muzzle black; tongue black; switch brown. Horns fair-sized, curving forward and in; white at base and black-tipped. Hoofs black; secretions yellow. Udder capacious, somewhat cut between quarters. Good-sized, tortuous veins, rather short. On the whole an excellent typical Jersey animal.

INDIVIDUAL DESCRIPTIONS OF THE CROSSBRED HERD

CROSSBRED 0: Lakeland's Poet (102603) × Delva Johanna De Kol (146774)—Jersey × Holstein-Friesian.

Male: Black, small amount of white; white spot on left front; elbow joint; white on brisket; small area in inguinal region. Muzzle and switch black; tongue black, with white spot in the middle of it. General type is that of the Holstein-Friesian. Horns heavy, coming straight out from head (Holstein-Friesian type). Four rudimentaries, one supernumerary placed on the scrotum.

CROSSBRED 1: Lakeland's Poet (102603) × Pauline Posch (81048)—Jersey × Holstein-Friesian.

Female: Black and white. No white on face; white spots on left side of brisket, extending as white streaks under forelegs; white spots flecked with black on left flank, irregular white areas on right side of udder. Muzzle black and white; switch black; interspersed with a few white hairs. Horns have white base, black tips, of medium weight, curving forward and in. Hoofs black; secretions light brown. General type that of a Holstein-Friesian × Jersey intermediate. The face and horns give more the impression of the Holstein-Friesian, for there is no dish in the face, and the body lacks the wedge shape of the Jersey. The lack of fleshing and small size of barrel show the Jersey influence. Udder pendent, with four poorly placed teats. Milk veins and wells rather small. Rather a low milker with a fair percentage of butter fat. Has a quiet dairy temperament.

CROSSBRED 2: Delva's University De Kol (133910) × Canada's Creusa (44386)—Holstein-Friesian × Guernsey.

Female. Black and white. Practically all black areas have some red on the base of the hair. White strip on face; white spot on shoulder connected on left side with a white belly, which extends as far forward as the end of the brisket. Forelegs white;

teats and udder white; white on hind legs, extending as an irregular area onto the flank. Tail about half white. Muzzle black, with white spot in the middle of it. Tongue white; switch white; horns white at base, black tips, turn upward and in. Hoofs white; secretions slight and dark brown. In general, the body type is that of a Holstein-Friesian. Udder of good size, but poor in shape; lacks filling in fore quarters, tending to be somewhat pendent. Four teats, one supernumerary on left side near the fore teat. Milk veins and wells of good size. Milk production rather large in quantity, but low in percentage of fat.

CROSSBRED 3: Johanna Lad Manor De Kol (41913) × Flora's Golden Poetess (264927)—Holstein-Friesian × Jersey.

Male. Black and white; white not distinctly separated from the black areas and small in the total amount. White on brisket; white spot on belly; right flank has three white spots; small white spot at base of switch. Muzzle light slate. Tongue is white with two small patches of black on left side and tip. Switch mixed black and white, black predominating. Horns those of the Holstein-Friesian, short, and curving in and upward. The type is intermediate between the Holstein-Friesian and the Jersey. In general the animal is too light for a Holstein-Friesian of the same age. Shoulders are heavy, fairly deep. Barrel is of medium size; rump long and level. Rudimentaries four and well placed.

CROSSBRED 4: Taurus Creamelle Hengerveld (98482) × Rosalie MSJHB (4457)—Holstein-Friesian × Jersey.

Male. Solid black, muzzle slate color, tongue a clay-blue, switch black. Horns of medium length, curving slightly forward. Type that of an intermediate, but giving more the impression of the Jersey than the Holstein-Friesian. This impression may be due to the evident quality and refinement which the animal exhibits. The size of bone is greater than that which may be expected of a Jersey bull of the same age, showing that, even in the question of quality, the Holstein-Friesian has had some influence. Four rudimentaries fairly well placed.

CROSSBRED 5: Kayan (167617) × Dot Alaska (29353)—Aberdeen-Angus × Ayrshire.

Male. Solid black except for a few white hairs on scrotum. Muzzle light slate. Tongue and switch black. Prominent scurs. Type in general that of the Aberdeen-Angus, although in the lateral view of the head, the straight nose, and large refined nostrils exhibit the Ayrshire character distinctly. Rudimentaries four and well placed.

CROSSBRED 6: Taurus Creamelle Hengerveld (98482) × Maple Grove Netta (29304)—Holstein-Friesian × Ayrshire.

Male. Black and white, the area of the white large in proportion to the black. Each white area has many black islands in it. The forehead has broad, V-shaped white star on it. Muzzle has a white strip. The throat is white. Upper part of forelegs and front of lower part white; white belly, the white extending across the shoulders. White on both sides of rump. Flanks and one side of hindlegs white. Muzzle slate-colored. Tongue flesh-colored; switch mostly white, but has some black in it. Type is that of an Ayrshire. Broad forehead, straight nose, clean-cut lips which are rather long. Horns large, coming straight out from head, turning slightly up. Four rudimentaries well placed (Pl. 3, C).

CROSSBRED 7: Kayan (167617) × Ruth 8th MSJHB (4457)—Aberdeen-Angus × Jersey.

Male. Solid black except for a white spot in the inguinal region. Muzzle medium slate; tongue pale clay-blue; switch black. No horns or scurs visible, although there are slight prominences under the hair. In general the type is that of an intermediate. From the front view the breadth and dish of the forehead, blockiness of shoulders and fore quarters, and the depth of barrel indicate the Aberdeen-Angus. In the side view the head is that of the Jersey, as it shows much dish. The hind quarters are too light

and thin for the Angus, and the whole body exhibits more the dairy type than the type of the beef animal. Rudimentaries four and well placed.

CROSSBRED 8: Kayan (167617) × College Creusa (25661)—Aberdeen-Angus × Guernsey.

Male. Solid color of a dark red-brown hue. Muzzle is brownish and smoky. Tongue a light slate. No horns or scurs present.

CROSSBRED 9: Kayan (167617) × Pauline Posch (81048)—Aberdeen Angus × Holstein-Friesian.

Male. Black and white. White extends from the region of the navel to the inguinal region as a narrow band. Muzzle and tongue black. Switch black, with a few white hairs scattered through it. Small loose scurs. Hoofs black; secretions brown. The head and fore quarters have a pronounced beef type. The hind quarters are not so heavily fleshed as one would expect from a beef animal of the same age. Rudimentaries four and well placed. Milk wells small; veins about 2 feet in length and of fair size (Pl. 4, A).

CROSSBRED 10: Kayan (167617) × Creusa of Orono 3d (34228)—Aberden-Angus × Guernsey.

Male. Brownish black except for a little white in front of scrotum. Muzzle and tongue light slate; switch black. No horns. The type is that of the Aberdeen-Angus, although it is much refined. The quarters lack something of the filling of the Aberdeen-Angus bull. Rudimentaries four, one small supernumerary placed in back of the hind rudimentaries about midway between them.

CROSSBRED 11: Lakeland's Poet (102603) × Delva Johanna De Kol (146774)—Jersey × Holstein-Friesian.

Female. Black and white; small star in middle of forehead. Lower part of brisket white, extending onto upper part of foreleg. There is a spot on the left foreleg and one on the right foreleg just above the knee. Upper part of belly has irregular white spots. Right side of udder white, left side of udder has the middle portion white and back part black. White spot on right flank. Lower part of tail above switch spotted with white. Little spot of white above ankle on each hind hoof. Muzzle slate; tongue white; switch white and black about equally mixed. Horns 7 inches, sharp, black tips, curving forward and in. Hoofs black; secretions a dark brown. The general type of body is that of a rather light Holstein-Friesian. In the side view of the face the thin nose and dish show clearly the Jersey influence. Four teats, one small supernumerary placed well up on middle of hind quarters (Pl. 4, B).

CROSSBRED 12: Taurus Creamelle Hengerveld (98482) × College Gem (40037)—Holstein-Friesian × Guernsey.

Female. Black, white, and gray-fawn. White on both forelegs and stockings extending to humerus. Belly has broad strip of white extending from between the forelegs as far back as the hind quarters of the udder. Hindlegs have stockings of a gray-fawn which extend as a regular line on right front side to the white on the belly. Muzzle black; tongue white; switch white; horns curved forward and slightly upward, with black tips. Hoofs black-and-white streaked; secretions orange. The type is essentially Holstein-Friesian in appearance, although the thinness of the face and the extremely heavy shoulders and high tail set show plainly the influence of Guernsey mother. The udder is of medium size, teats four and fairly well placed. Quality fair.

CROSSBRED 13: Columbia's Fox (126386) × Eventime 4th (55526)—Jersey × Aberdeen-Angus.

Male. Black, with a small amount of white on underside of throat and in the inguinal region. Muzzle slate; tongue light clay-blue; switch black. Fair-sized scurs. The breadth and height of forehead and heavy shoulders indicate the Aberdeen-Angus. The thin, narrow nose, low heart girth and lack of body depth, hair, and evident quality resemble the Jersey. However, the evident roundness of build,

and deepness of fleshing make the general type too much that of a beef animal to favor the Jersey. Four well-placed rudimentaries.

CROSSBRED 14: Taurus Creamelle Hengerveld (98482) × Flying Fox's Flora (274051)—Holstein-Friesian × Jersey.

Male. Black and white. White between the forelegs extending as irregular spotting on either side into the inguinal region. Two white spots on right shoulder; white spot on right hip and right flank. White at base of switch. Muzzle white; tongue black; switch black-tipped white coverts. Horns white, black-tipped. Hoofs black; secretions light brown. Intermediate in type between the Holstein-Friesian and Jersey. Rudimentaries well placed; milk veins and wells small, about 20 inches on either side (Pl. 4, C).

CROSSBRED 15: Lakeland's Poet (102003) × Hearthbloom (147141)—Jersey × Aberdeen-Angus.

Female. Solid black. Muzzle black; tongue slate; switch black. Horns slight stubs just through the skin. Hoofs black; secretions a dark orange bordering on brown. Head, shoulders, and withers give the appearance of the Aberdeen-Angus except that they are somewhat reduced in size. The fleshing of the rest of the body is rather heavy, but not heavy enough for the beef type. In general it may be said that the barrel, rump, flanks, and twist are of the Jersey type, which is heavily fleshed. Four well-placed teats; milk veins and wells small. Quality good. The poll of the head has the peculiar knobbed appearance which is characteristic of the Aberdeen-Angus (Pl. 5, A).

CROSSBRED 10: Kayan (107017) × College Ruth MSJHB (4805)—Aberdeen-Angus × Jersey.

Female. Solid black. Muzzle black; tongue slate; switch black. No horns present, although there are slight loose prominences under the skin. Hoofs black; secretions dark brown. The face is intermediate in type between the Jersey and Aberdeen-Angus. In the front view of the face the great breadth of forehead and nose of the Aberdeen-Angus are not seen. In the side view the face lacks the dish of the Jersey. The general type is intermediate between the two breeds, the fore quarters favoring the beef type, the barrel, rump, and hind quarters generally indicating the Jersey type, which has a considerable amount of flesh on it. Teats four, fairly well placed.

CROSSBRED 17: Kayan (167617) × Rue Victoria (273096)—Aberdeen-Angus × Jersey.

Male. Black, except for a small amount of white in front of the inguinal region. Muzzle black; tongue black; switch black. Loose scurs about 1 inch in length. Hoofs black; secretions brown. Head, shoulders, and withers resemble those of a light Aberdeen-Angus bull. The depth and length of body and rump show the characteristics of a Jersey quite heavily fleshed. Loin has more breadth and filling than would be expected of a Jersey of the same age. Milk veins about 14 inches on each side. Rudimentaries four and fairly well placed. Quality fair.

CROSSBRED 18: Kayan (107017) × Ruth 8th MSJHB (4457)—Aberdeen-Angus × Jersey.

Male. Twin to No. 19. Black in color. Died when only a day old.

CROSSBRED 19: Kayan (107017) × Ruth 8th MSJHB (4457)—Aberdeen-Angus × Jersey.

Female. Twin to No. 18. Solid black. Muzzle, tongue, and switch black. No horns, although slight, loose concrescences can be felt through the skin. Hoofs black; secretions brown. Face, shoulders, and fore quarters resemble the Aberdeen-Angus. Hind quarters are those of the Jersey in very high condition. Hair rather coarse in quality like that of its father. Four well-placed teats; milk veins small but long, leading to four wells (Pl. 5, B).

CROSSBRED 20: Taurus Creamelle Hengerveld (98482) / Maple Grove Netta (29304)—Holstein-Friesian × Ayrshire.

Male. Black and white. Broad white star on forehead. Throat white; white band extending across shoulders onto belly on the left side. White spot on right shoulder. White on dewlap and brisket. Front side of forelegs white; back black and white spotted. Belly black and white, white extending as an irregular band to the region of escutcheon. Spot on right flank; irregular white area extending down onto left leg from tail. Hindlegs white on front side; backside black. Line of demarcation between white and black irregular. Muzzle black; tongue white; switch white. Horns large, rather thick at base, extending upward and in, as a characteristic of the Ayrshire type. The general type of body is that of an intermediate, tending more toward the typical Scottish Ayrshire build. However, the thickness of the shoulders and the depth of fleshing show the Holstein-Friesian blood. Four rudimentaries and two supernumeraries placed near left hind rudimentary.

CROSSBRED 21: Kayan (167617) × Dot Alaska (29353)—Aberdeen-Angus × Ayrshire.

Male. Black except for a very small area of white in the inguinal region. Muzzle dark slate; tongue black-tipped, with a small spot of black on right side; otherwise white. Switch black. Horns large and quite solidly attached. The face and body generally are those of the Aberdeen-Angus, although the horns and V-shaped appearance of the head show the stamp of the Ayrshire. Fore quarters are distinctly Aberdeen-Angus, hind quarters are a trifle high in twist, and perhaps a little light for this breed. Four large, prominent rudimentaries well placed (Pl. 6, B).

CROSSBRED 22: Kayan (167617)×College Creusa (25661)—Aberdeen-Angus×Guernsey.

Female. Black and white; white streak on belly and around udder. Muzzle black. Tongue slate; switch black. Horns are entirely absent. Hoofs black; secretions brown. The face tends more to the Guernsey than to the Aberdeen-Angus type, except that the horns are lacking. Withers and brisket heavy, barrel well rounded, poorly fleshed for the Aberdeen-Angus. On the whole, the general appearance is more that of a Guernsey, which is very low set, than it is that of the Aberdeen-Angus. Teats four, well placed; milk veins and wells small. Veins are too fine for them to be felt through the thick coat.

CROSSBRED 23: Taurus Creamelle Hengerveld (98482)×Columbia Brown Bessie (148551)—Holstein-Friesan×Jersey.

Male. Black and white. White spot on forehead; white on brisket; white in inguinal region; and white stockings on hind feet. Muzzle black; tongue white, with a small black spot on tip of it. Switch white. Horns well developed, curving forward and slightly up. The general type is that of an intermediate, favoring the Jersey in the side view of the head and light-fleshed hind quarters. The shoulders and withers are those of the beef breed, although not carrying quite as much fleshing. Rudimentaries four and well placed (Pl. 5, C).

CROSSBRED 24: Kayan (167617)×College Creusa 2nd (34227)—Aberdeen-Angus×Guernsey.

Male. Black, except for white shield in inguinal region. Muzzle dark slate; tongue dark slate; switch black. Horns small and easily movable, showing that they are not attached to the bone of the head. The contour of the face is intermediate, but favors that of the Aberdeen-Angus. The body is more Guernsey than Aberdeen-Angus in type, although the heavy fleshing of the fore quarters and shoulders and rather deep flesh of the hind quarters show the beef type. The rudimentaries are four, the two front ones being quite small. All four are rather well placed.

CROSSBRED 25: Crossbred 0×Crossbred 1—Jersey-Holstein-Friesian × Jersey-Holstein-Friesian.

Female. Color black and white. Broad white star on forehead; white at throat, irregular broad white band extends over withers from the right side and connects with the belly on the left side. Brisket white; left foreleg has white stocking, and the other side is white. Belly white as far as the escutcheon. Hindlegs white. Irregular white spot including the rump and tail base. The lower extremity of the tail white. Muzzle black; tongue flesh-colored; switch white. Horns curved forward and in, rather light, and about 5 inches long. Hoofs black; secretions brown. The animal is, on the whole, rather small for her age. She exhibits the Holstein-Friesian type in the face when viewed from front or side. Shoulders and chest rather heavy and deep. Four fair-sized, well-placed teats.

CROSSBRED 26: Kayan (167617)×Creusa of Orono 3d (34228)—Aberdeen-Angus× Guernsey.

Female. Color solid black, except for a small white area around the teats. Muzzle black; tongue slate; switch black. No horns nor scurs present. Hoofs black; secretions yellow. In general, the face and shoulders favor the Aberdeen-Angus type. The barrel and hind quarters, which are quite heavily fleshed, show plainly the Guernsey character. The back is somewhat swayed, like the back of the Guernsey mother. Teats medium-sized, not especially well placed.

CROSSBRED 27: Lakeland's Poet (102603)×Orono Madge (192781—Jersey×Aberdeen-Angus.

Female. Black, with a small amount of white around the teats. Muzzle black; tongue slate; switch black. No trace of horns nor scurs present. Hoofs black; secretions yellow. The type of the face is intermediate, although rather favoring the Jersey, even while it still retains some of the breadth and lacks the dish characteristic of this breed. Shoulders are heavy and deeply fleshed. Barrel and rump give the appearance of a Jersey animal in high condition. Back is considerably swayed, this characteristic coming probably from the Jersey father. Teats four, of medium size, and rather well placed.

CROSSBRED 28: Kayan (167617)×Pauline Posch (81048)—Aberdeen-Angus× Holstein-Friesian.

Male. Black, except for a small white spot on sheath and scrotum. Very little white area on right hind leg. Muzzle black; tongue black on distal half, proximal half white; switch white. The horns are represented by small scurs shown not to be attached to the bone of the head, since they are movable. Face and fore quarters show the Aberdeen-Angus characters plainly. Hind quarters are intermediate, but favor the Holstein-Friesian, as the animal stands too high on its feet and is too highly cut up in the twist for a good Aberdeen-Angus. Four rudimentaries are present, the front ones being ¾ inch long.

CROSSBRED 29: Kayan (167617) × Creusa's Lady (53234)—Aberdeen-Angus × Guernsey.

Female. Black, brown, and white. Brown ring around eyes and on inside of fore and hind leg. White line along underside of belly. Muzzle white; tongue slate in distal, flesh color in proximal portion; black switch. No horns present. Hoofs black; secretions brown. Head narrow and thin, long for the Aberdeen-Angus; only a slight dish. The shoulders are rather heavy tending more toward the beef form than toward the dairy type. Body and hind quarters resemble the Guernsey. Four teats fairly well placed.

CROSSBRED 30: Taurus Creamelle Hengerveld (98482) × Orono Ellen (192783)—Holstein-Friesian × Aberdeen-Augus.

Male. Black and white. White extending as a line on the belly as far as the brisket. Coronets of both hind feet white. Muzzle black; tongue black; switch black and white, black predominating. Loose scurs about 1 inch in length. Hind hoofs white; front hoofs black; secretions light brown. Lower part of the face resembles the Holstein-Friesian; upper part has the character of an Aberdeen-Angus. The type of

the rest of the body is that of an intermediate, resembling the Aberdeen-Angus in the increased amount of flesh carried by the animal. Four rudimentaries fairly well placed. Animal shows a good deal of quality.

CROSSBRED 31: Lakeland's Poet (102603) × Eventime 4th (155526).

Male. Solid black. Muzzle black; tongue black; switch black. Horns are loose. Hoofs black; secretions considerable in amount and a dark orange. Face is rather long, narrow, and more on the order of the Jersey than the Aberdeen-Angus. Shoulders rather heavily fleshed. Four fairly well placed rudimentaries. Mammary vein small. Quality is rather good.

CROSSBRED 32: Kayan (167617) × Canada's Creusa (44386)—Aberdeen-Angus × Guernsey.

Male. Black except for a small area of white around rudimentaries. Muzzle black; tongue black; switch black. Horns of good length and solid. Hoofs black; secretions light brown. The head and shoulders are essentially those of an Aberdeen-Angus thinly fleshed. The rest of the body indicates the type of a Guernsey bull of about the same age. Rudimentaries rather small, placed close together.

CROSSBRED 33: Lady Primrose Governor of the Fountain (18328) × Hearthbloom (147141)—Guernsey × Aberdeen-Angus.

Female. Solid brownish black. Muzzle slate; tongue a light slate; switch black. The front view of the face resembles the Aberdeen-Angus with the exception of having a rather small muzzle. The side view shows the influence of the Guernsey. On the whole the animal is much too blocky of form to resemble a typical Guernsey, and all in all resembles the paternal side rather than the maternal in its shape of body.

CROSSBRED 34: Crossbred o × Rosalie MSJHB (4887)—Jersey – Holstein-Friesian × Jersey.

Female. Red and black. Light fawn on underside of throat. Neck gives a dark-red appearance, owing to the under hair being a red and the outer hair nearly black. Barrel has a dark reddish tinge, black on front side of foreleg and red on the back side. Hindlegs a red-fawn. Muzzle black; tongue black; switch black with a few hairs in it. Horns of a medium length. Hoofs black; secretions orange. The head and body give the characteristics typical of the Jersey. Four well-placed rudimentaries of fair size.

CROSSBRED 35: Taurus Creamelle Hengerveld (98482) × Flying Fox's Flora (274251)—Holstein-Friesian × Jersey.

Male. Black and white. White on belly. Short white stockings on forelegs; hindlegs have rather long stockings. Muzzle black; tongue white; switch white. Horns rather long, solidly attached. Hoofs white; secretions a light brown. The face is that of a Jersey somewhat shortened and increased in breadth. Barrel and hind limbs are Jersey except that withers are rather heavy. Four rudimentaries.

CROSSBRED 36: Kayan (167617) × Orono Netta (38832)—Aberdeen-Angus × Ayrshire.

Male. Solid black. Muzzle black, tongue slate. The type is that of an intermediate throughout. The shoulders and body are rather more thick-set and blocky than would be expected of the Ayrshire and a little too rangy for the Aberdeen-Angus.

CROSSBRED 37: Kayan (167617) × Dot Alaska (29353)—Aberdeen-Angus × Ayrshire.

Female. Black and white, the white occurring as a white irregular patch around the teats. Muzzle black; tongue black; switch black. No trace of horns. Hoofs black; secretions dark brown. Face is an intermediate between the Ayrshire and Angus, leaning more toward the Aberdeen-Angus type. Body distinctly Ayrshire except for the shoulders, which are much too heavy for that breed. Teats good size, fairly well placed. Udder of good size, quality fair.

CROSSBRED 38: Crossbred 10 × College Creusa (25661)—Aberdeen-Angus–Guernsey × Guernsey.

Male. Solid color, a dark shade of orange, a little lighter on legs and hindlegs. Middle is really smutted with black, tongue is cream-colored, switch red. Scrotum is a light orange. Face is that of a Guernsey except for the fact that it is rather short and broad. Has some fish. Shoulders are a little heavy, but aside from that the animal looks beefy (the type which would be expected of a Guernsey bull of the same age. Rudimentaries small, fairly evenly placed. Pl. 6, A).

Crossbred 39: Kayan (10020) \ Rue Victoria (17902)—Aberdeen-Angus \ Jersey.

Male. Solid black. Muzzle black; tongue slate; switch black. Scurs are short and loose. Hoofs black, secretions brown. The face is plainly that of the Aberdeen-Angus, and the general type of body is intermediate between the two breeds. Four well-placed, good-sized rudimentaries.

Crossbred 40: Kayan (10020) \ College Cream and (54887) — Aberdeen-Angus \ Guernsey.—

Male. Black and white, white limited to a small area around the rudimentaries. Muzzle black; tongue slate; switch black. Horns consist of small scales just through the skin. Hoofs black, secretions brown. The forehead and shoulders are more those of the Aberdeen-Angus than those of the Guernsey. A thin muzzle and rather lighter fleshing of the rest of the body give the appearance of a beef and milk animal rather than that of either the beef or dairy type. Rudimentaries four, about equal distance apart.

Crossbred 41: Kayan (10020) \ College Gem and (52455) — Aberdeen-Angus \ Guernsey.

Female. Solid brownish black. Muzzle black; tongue slate; switch black. No evidence of horns, but it really died too young to determine this point. Face gives a general appearance of the Guernsey type. Fore quarters barrel, and hind quarters intermediate.

Crossbred 42: Crossbred a \ Flora's Golden Poetess (10990) — Jersey-Holstein-Friesian \ Jersey.

Female. General color a dark fawn with no white spots. The dark-colored hair intimately mingled with a smaller proportion of black hair. Muzzle black; tongue black; switch black. Horns present. Hoofs black, secretions brown. The general build of the face and general body contour tends toward the Jersey type rather than the Holstein-Friesian.

Crossbred 43: Crossbred a \ Crossbred 1 — Jersey-Holstein-Friesian \ Jersey-Holstein-Friesian.

Male. Four white spots on the front side of both forelegs. White brisket, irregular white spots on belly made up of four white streaks on both sides. Muzzle black; tongue white with a black spot in it toward the base; switch black. Short horns present and hoofs black. General type of both the body and face is that of a Holstein-Friesian.

Crossbred 44: Taurus Creamelle Hengerveld (16482) \ Dewdrop Madge (10078)—Holstein-Friesian \ Aberdeen-Angus.

Female. Solid black. Muzzle black; tongue black; switch black. Very small, loose scurs present. Hoofs black. The face is narrow and thin, without much flesh, indicating the Holstein-Friesian type. The fore quarters are Aberdeen-Angus. The body and hind quarters are intermediate, favoring slightly the Aberdeen-Angus type.

Crossbred 45: Kayan (10020) \ Pauline Posch (51048) — Aberdeen-Angus \ Holstein-Friesian.

Female. Black, with a small white streak on the belly. Muzzle black; tongue, distal portion black, proximal white; switch black. Hoofs black. General type is that of an intermediate, with the face and fore quarters favoring more the Aberdeen-Angus.

Crossbred 46: Taurus Creamelle Hengerveld (16482) \ Lassie of M. F. (10030)—Holstein-Friesian \ Jersey.

Female. Black and white. White belly to brisket. Short white stockings. Lower part of tail white. Muzzle black; tongue slate in the distal portions and white in proximal; switch white. Large horns present. The face is of the extreme Jersey type, almost a complete reproduction of that of her mother. The rest of the body is intermediate between the two breeds.

CROSSBRED 47: Taurus Creamelle Hengerveld (98482) × Hearthbloom (147141) — Holstein-Friesian × Aberdeen-Angus.

Female. Black and white, with small white areas extending the length of the belly. Muzzle black; tongue black-tipped, white at base; switch white-tipped. Hoofs black; secretions dark. The general type of the body and face is that of an Abdereen-Angus.

CROSSBRED 48: Crossbred 0 × Crossbred 11 — Jersey–Holstein-Friesian × Jersey–Holstein-Friesian.

Male. Black and white. Small star on forehead. Irregular white spot on left shoulder. Irregular white areas on both fore feet. Small white spot on left hind ankle. Belly white; muzzle black; tongue white; switch white. Horns present. Hoofs black. General type is that of a Jersey.

CROSSBRED 49: Taurus Creamelle Hengerveld (98482) × Crossbred 2 — Holstein-Friesian × Holstein-Friesian–Guernsey.

Male. Black and white. White under throat and on underside of neck. Fore and hind legs white; belly white; muzzle white, with four black spots scattered over it; tongue white; switch white. General appearance is that of a Holstein-Friesian.

CROSSBRED 50: Taurus Creamelle Hengerveld (98482) × Eventime 4th (155526)— Holstein-Friesian × Aberdeen-Angus.

Male. Black and white. Large star. White spot on left shoulder and irregular white spots on both flanks. Belly white to brisket; muzzle black; tongue slate at tip, with white in proximal half; switch white. This animal died when it was too young to determine whether or not horns would be present. Hoofs black in the fore parts and white behind. The general type is that of an intermediate between the two breeds.

CROSSBRED 51: Taurus Creamelle Hengerveld (98482) × Crossbred 12—Holstein-Friesian × Guernsey–Holstein-Friesian.

Male. Black and white. Medium-sized star. White belly; fore and hind feet white; muzzle black; tongue white; switch white. Horns present. Hoofs white. The general type of the body is that of the Holstein-Friesian.

CROSSBRED 52: Taurus Creamelle Hengerveld (98482) × Orono Ellen (192783)— Holstein-Friesian × Aberdeen-Angus.

Female.—Black and white, the white limited to the inguinal region. Muzzle black; tongue black; switch black. The general type of the face aud fore quarters is that of the Aberdeen-Angus, the hind quarters resembling the Holstein-Friesian.

CROSSBRED 53: Taurus Creamelle Hengerveld (98482) × Rosalie MSJHB (4887)— Holstein-Friesian × Jersey.

Male. Solid black. Muzzle black; tongue black, with a small white spot near its base; switch black. Horns present. The general appearance of the face and body is that of the Jersey type.

SPECIFIC PROBLEMS FOR ANALYSIS

These data just presented offer perhaps the most accurate scientific material on first-generation crosses that exists for the study of many vexing questions on inheritance in cattle. Their collection is the result of a carefully planned series of experiments both to test the result of other investigations and to bring forth new data on the subject. The

descriptions of the crossbreds and their parents furnish the material for the objects for our preliminary study—

(1) To determine the inheritance of the following color characters: Body colors of the dairy cattle, red, white, yellow, fawn (in its various shades), and black; the white markings, muzzle and tongue pigment, and switch color.

(2) To determine the mode of inheritance of the polled character.

(3) To determine the inheritance of the body and breed type in the first generation.

(4) To determine whether quantity and quality of milk are characters which exhibit dominance and segregation.

EXPERIMENTAL RESULTS

INHERITANCE OF COLOR CHARACTERS

Body Color

The summary of the data for the individual description is tabulated as follows: The offspring of a given bull are given under the name of the bull. The description of the mating consists of, first, the description of the character studied in the given bull and the description of this same character in the dam. Following these parental descriptions are given the description and number of a given kind of offspring resulting from a given mating. This same scheme of tabulation of the results is used for all characters studied in the paper. The term "solid color" is used in the following sense: In speaking of the general body color of an animal it means that that animal has no white markings whatsoever. In speaking of a given part of the animal, as the face, it means that this part has no white markings, although white may occur in other places throughout the coat. The term "color" is considered to be the ground color of the body and not the white spottings. With these new definitions made clear the consideration of the data on the behavior of the coat color in inheritance for our crossbred herd may be taken up (Table VII).

Table VII shows that black body color is dominant to all other colors, red, brown, and fawn. Two interesting cases of segregation occur. The deep-orange-coated bull and the dark-fawn heifer are shown to come from matings of black F_1 parents. These cases are too few to base any definite conclusions as to the number of factors in coat-color inheritance, but what they do indicate is that there is a particular inheritance and not a blending one.

These conclusions are in line with the general experiences of those who have bred black cattle and fawn or red colored animals together. Spillman (*41*) showed that the progeny of F_1 hybrids of black on red cattle behaved in Mendelian fashion with red recessive. This conclusion was further emphasized by the crosses made of the Hereford on Aberdeen-Angus by Boyd (*10*), in which he showed that this black of

the Aberdeen-Angus was dominant. The red of the Hereford segregated out in the F_2 generation. Further, in his interesting crosses of bison on domestic cattle, Boyd (*10*) shows that the bison on almost any breed produced either black or brindle body color, indicating that here also the dark body color was dominant.

Early in the study of the inheritance of color in cattle Barrington and Pearson (*6*) collected data to show that red cattle sometimes resulted from the cross of two black Galloways. This was used by them as an argument against the Mendelian explanation. It remained for Lloyd-Jones and Evvard (*20*) on their own data and that of Youatt (*59*) to show that this was no anomaly to the Mendelian explanation, but that it was due to the grade Galloways being heterozygous for this red. In some crosses of Shorthorns on Galloways they show that in the straight F_2 generation a clean-cut segregation of 20 blacks to 6 reds was obtained.

TABLE VII.—*Behavior of the general body color in the parental and first filial generations of cattle*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld	Black × black	7 black.
Do	Black × fawn	5 black.
Do	Black × F_1 black carrying fawn.	2 black.
Kayan	Black × black	3 black.
Do	Black × fawn	15 black.
Do	Black × red	4 black.
Lakeland's Poet	Fawn × black	6 black.
Crossbred o F_1	F_1 black carrying fawn × fawn	1 black, 1 dark fawn, some black hairs present.
Do	F_1 black carrying fawn × F_1 black carrying fawn.	3 black.
Crossbred 10 F_1	F_1 black carrying fawn × fawn	1 deep red-fawn.
Lady Primrose's Governor of the Fountain.	Fawn × black	1 black.
Delva's University De Kol	Black × fawn	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Fawn × black	Do.

The F_2 generation of our crosses contains two very interesting animals as above mentioned: One, a deep-orange bull, was the result of a back-crossed black F_1 bull carrying the Guernsey color onto a Guernsey. The other was produced by a back cross of a black F_1 bull, Holstein-Friesian × Jersey, onto a dark Jersey. The coat of this heifer was very dark. The appearance of the dark-fawn and deep-orange offspring from black F_1 parents indicates that segregation of the parental coat colors does occur. The deep-orange bull is of especial interest since he shows the segregation of the Guernsey coat with the color modified. This deepening of the shade of the coat seems to be confirmatory evidence for the hypothesis advanced by Wright (*58*) that the coat color of Guernsey cattle differs from the other dun-colored breeds by a reces-

sive dilution factor for this fawn. The proof for this factor being recessive comes in the fact that the F_1 cross was a back cross of a black Guernsey F_1 bull on a Guernsey cow. Furthermore, this factor from the nature of the cross can not be sex-linked as in the case of Wentworth's explanation of the dark shade of brown in the coat of the male Ayrshire (*40*).

The case of the almost identical appearance of the dark dun F_2 heifer and her Jersey parent agrees well with the findings of Kuhlman (*17*) for Jersey × Aberdeen-Angus crosses. These cases support the conclusion that in the Jerseys any such dilution factor as that in the Guernsey is not normally present.

General White Markings of the Coat

Most investigators have considered that white markings found in the different parts of the coat were due to a piebald factor exhibiting great somatic variation (*19*, *21*, *43*, *48*). This statement of the case seems to the author to have many contradictions, but as this appears to be the popular belief the data will be treated first from this point of view. Table VIII presents this treatment.

Table VIII.—*Behavior of the general white markings in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Spotted × solid color	2 solid colors, 5 spotted.
Do	Spotted × spotted	8 spotted, 1 solid color.
Kayan	Spotted × solid color	3 solid color, 3 spotted.
Do	Spotted × spotted	3 solid color, 13 spotted.
Lakeland's Poet	Solid color × solid color	1 solid color.
Do	Solid color × spotted	2 solid color, 3 spotted.
Minor crosses:		
Crossbred 0	F_1 spotted carrying solid color × solid color.	2 solid color.
Do	F_1 spotted carrying solid color × F_1 spotted carrying solid color.	3 spotted.
Crossbred 10	F_1 spotted × spotted	1 solid color.
Lady Primrose's Governor of the Fountain.	Spotted × spotted	Do.
Delva's University De Kol.	do	1 spotted.
Johanna Lad Manor De Kol.	Spotted × solid color	Do.
Columbia's Fox	do	Do.

Two points of importance come out in consideration of these data: First, the white piebald factor must be dominant; second, this factor must be present in the bulls in the heterozygous form, as each bull throws some solid-color animals. The first conclusion is not strange, for it is to be expected that the factor is dominant, recessive, or perhaps intermediate in its effect. The fact could, however, be just as well explained by the presence of a dominant factor for white in a given part of the

body and the simultaneous presence of other recessive factors for white in the rest of the body. The second conclusion could be explained on chance sorting, in which chance had given us nine heterozygous bulls. Such a conclusion would seem unlikely, unless the piebald condition is lethal when homozygous. Furthermore, the piebald condition is always favored in the ratios obtained as this work and that of Kiesel (*18*) have shown.

The work of others furnishes further supporting evidence for the view that the white markings are brought about not by one factor but by the interaction of many. Boyd (*10*) and Nabours (*26*) have shown that the white face of the Hereford is dominant. In collecting evidence for cases parallel to their case of animals white with red and with black ears Lloyd-Jones and Evvard (*20*) found Storer (*20*), Wilsdorf (*48*), and others had described similar cases. Chillingham and Park cattle have such dominant markings of white body coat with black ears; and Pembroke and Highland cattle, which have a coat of almost identical pattern, have the character recessive. Wilson (*51*) further fills in the gap by describing cases of cattle which were all white. Besides these examples the belt of the Dutch-belted cattle has been shown to be separately inherited. Thus it would seem that there are many cases where a given kind of white is separately inherited.

All of these cases are important for the interpretation of the coat of the roan Shorthorn. Already some of those differences in behavior of the white have been used for parallels to explain the peculiar ratios obtained in these Shorthorn cattle studies (*20*, *58*). It remains, however, to be shown that there is any difference in the inherited white throughout the different parts of the coat before this parallelism is established. Allen (*1*) has made a beginning at this kind of attack by his studies on the distribution of these white markings in mammals and birds. His studies as to the location of the centers of these spots are in general found to check well with this study. The location of the areas may be given as follows: The forehead, the throat, a band across the shoulders often extending to the belly, an irregular spot on the rump including some of the tail set, irregular spots on the flanks, lower half of tail above switch, belly from brisket to inguinal region and any leg as a coronet, or as a short or long stocking.

The data on these crossbreds as above presented are well suited to this novel analysis. The markings of both parents and offspring have been carefully examined and described in writings, by drawings, and with photographs. The areas were found to fall naturally into the groups above mentioned. The material to determine the behavior in inheritance of the various groups is available.

The general practice followed throughout the study of these individual spots is to consider each area separately. The general conclusions for the behavior of these areas are then given. If there are any

cases which appear anomolous to the conclusion, these are considered together with their color pedigree. The summary and general conclusions are reserved for the end of this section on white markings.

WHITE MARKINGS OF THE FACE

The markings of the face have been divided into the classes familiar to most breeders: Star, star and snip, blaze, and solid color. In all of these studies the presence of a few white hairs is considered as star or snip, etc., according to the place of its appearance.

The behavior of the F_1 progeny markings is shown in Table IX.

TABLE IX.—*Behavior of the white markings on the face in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Star × solid color	8 solid + 3 star.
Do	Star × star	1 solid + 1 star, 1 star snip.
Do	Star × F_1 blaze carrying star.	1 blaze.
Do	Star × F_1 solid color carrying star.	1 star.
Kayan	Solid color × solid color	9 solid color.
Do	Solid color × star	10 solid color.
Do	Solid color × blaze	3 solid color.
Lakeland's Poet	Solid color × solid color	Do.
Do	Solid color × star	1 solid color + 1 star.
Do	Solid color × blaze	1 solid color.
Minor crosses:		
Crossbred 0	F_1 solid color carrying star × solid.	2 solid color.
Do	F_1 solid color carrying star × F^1 solid carrying star.	2 star, 1 solid color.
Crossbred 10	F_1 solid carrying star × star.	1 solid color.
Lady Primrose's Governor of the Fountain.	Star × solid color	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Solid color × solid color	Do.

As a whole, Table IX indicates that white markings of the face are suppressed in the F_1 offspring when one of the parents is solid color. There is one exception to this interpretation. Heifer 12 had no mark and came from Taurus Creamelle Hengerveld (large star) and College Gem (very small star). It may be, of course, that this star in College Gem is hair just turning gray with age. This does not seem likely, however, and it seems best to consider this a case against so simple an hypothesis as a single recessive. Four other cases offer exceptions if it should prove true that their solid-color parents are genetically solid colored. Unfortunately, only two of these cases can be pedigreed, as they alone come from breeds recording color markings as part of their registration for the herd book. The first of these (Columbia's Brown Bessie) has her color pedigree given in Table X.

The pedigree of this animal has so much recorded white in it that it is possible to make the appearance of white on any of her offspring quite likely from the standpoint of chance inheritance of single factors when she is mated to white bulls.

TABLE X.—*Pedigree of Columbia's Brown Bessie*

Maine Agr. Exp. Sta.—Standard Pedigree Blank.

Sex ♀				
COLUMBIA'S BROWN BESSIE.				
No. 148551.	♂ No. 47278. Brown Bessie's Son 6th. Solid color, black tongue and switch.	No. 34550. ♂ Brown Bessie's Son. Solid color, black tongue and switch.	No. 17576. ♂ Combination 3d. Solid color, black switch.	No. 4389. Combination. ♂ Solid color, black points.
				No. 29330. Coma. ♀ Solid color, black tongue and switch.
			No. 74997. ♀ Brown Bessie. White flanks, belly, and tongue; switch black.	No. 7890. Voleo. ♂ Solid color, white tongue, black switch.
				No. 74996. Brown Flora. ♀ Solid color, black tongue and switch.
		No. 37496. ♀ Fancy Bee. Star, white flanks; fleck on right thigh; white armpits, belly, forefeet, and hind socks, and switch.	No. 9777. ♂ Fancy's Harry. White forefeet; white hind stockings; white tongue; mixed switch.	No. 3445. Lord Harry. ♂ Solid color.
				No. 2876. Landseer's Fancy. ♀ Eyelids buff; star; white on shoulder; white forefeet, hind legs; belly white.
			No. 9807. ♀ Beeswax. Gray; white in both flanks, left hip, both hind legs, and left forefeet; white belly.	No. 1404. Top Sawyer. ♂ Solid squirrel gray; black tongue and switch.
				No. 1870. Besma 3d. ♀ Solid color, black tongue, hoof, and switch.
	♀ No. 103191. Columbia's Fancy. Solid color, dark tongue, and black switch.	No. 26853. ♂ Turbigo's Best Son. Solid color, black tongue and switch.	No. 18208. ♂ Turbigo. Solid color, black tongue and switch.	No. 3533. Tormentor. ♂ Gray; dark shading; small star; white belly, front of hind legs, and fore ankles.
				No. 24757. Majestic. ♀ Solid color, white tongue, and brown switch.
			No. 49119. ♀ Zoranda. White spot on each flank, on right thigh; white belly; white tongue and switch.	No. 15664. Ruby's Harry. ♂ Spot on face; white on right arm, flanks, belly, and left hind leg; right hind stocking white; mixed switch.
				No. 10592. Miss Everdeen. ♀ Partly colored.
		No. 80927. ♀ Flossie Landseer. White fleck on each flank, armpits, and belly; white on thigh; hind legs, tongue, and switch white.	No. 24886. ♂ Fancy's Harry 7th. Mixed black and white switch; white tongue.	No. 9777. Fancy's Harry. ♂ White on forefeet; white hind stockings; white tongue; mixed switch.
				No. 32989. Ruby Torment. ♀ Little white on brisket and flanks; white belly; white on legs; white switch.
			No. 75581. ♀ Aletha of Mount Pleasant. Solid color, black tongue and switch.	No. 11578. Sir Florian. ♂ Solid color, black tongue and switch.
				No. 33487. Enna. ♀ Solid color, black tongue and switch.

Fancy Bee (37496), the granddam on the father's side, had a star which, if the hypothesis that the star is due to a single recessive is correct, would have been transmitted to Brown Bessie's Son 6th in the heterozygous form (single dose). This gives an even chance that Columbia's Brown Bessie carries a star.

The case of Lakeland's Poet, father of Heifer 11, is not quite so good. There is still, however, a good chance that he carries this star, as may be seen from his color pedigree (Table XI).

TABLE XI.—*Pedigree of Lakeland's Poet*

Maine Agr. Exp. Sta.—Standard Pedigree Blank.

No. 102603. LAKELAND'S POET. Sex ♂			
No. 214093. Spot on each forerankle and knee; white brisket; light tongue and mixed switch. ♀ NORA OF MONMOUTH 2D.	No. 65780. Black tongue and switch; some white on brisket; speck on left side of belly, and patch on belly near left flank. ♂ MABEL'S POET.	No. P. S. 2591. ♂ CAIEST. White under chest; white on forelegs; switch white.	No. 2207. ♂ S. FONTAINE'S KING. White on each stifle; tail and tongue white.
			No. 1559. S. BOYLE. ♂ White spot on forehead; tail and tongue white.
			No. 2537. D. FONTAINE 4TH. ♀ Fawn and white; white patch on forehead; white spot behind right shoulder.
		No. 3582. ♀ D. OXFORD LASS. White across withers; tongue black; tail white.	No. 928. COUNT WOLSELEY. ♂ Brown, solid color; tail and tongue black.
			No. 2252. D. OXFORD PRIMROSE. ♀ Fawn; white stripe on left shoulder.
		No. P. S. 6311. ♀ MABEL 35TH. Solid color, red muzzle.	No. 1559. ♂ S. BOYLE. White spot on forehead; tail and tongue white.
			No. 1242. S. GOLDEN LAD. ♂ Gray-fawn, solid color; tongue and switch black.
			No. 3214. D. TOOTSIE. ♀ Gray; white on each stiffle; white ring in tail; tongue white.
		No. 3213. ♀ D. MABEL 23D. Tongue and tail black dappled on chine.	No. 390. S. EVERTON KING. ♂ Gray, solid color; tongue black and white.
			No. 1125. D. MABEL 13TH. ♀ Brown; white spot on loins; white speck behind each shoulder; tail black.
		No. 53844. ♂ PEDRO'S POLO. Solid color, black tongue and switch.	No. 45486. ♂ GENERAL MARIGOLD. Solid color, black tongue and switch.
			No. 34969. MAJOR POLO. ♂ Solid color; brown tongue and switch.
			No. 88186. MARY IDAGOLD. ♀ Solid color except little white on belly; black tongue and switch.
		No. 88543. ♀ RIOTERS PRETTY BELLE. Solid color except belly; hind legs, tail, and switch white; white tongue.	No. 26015. PEDRO OF LINDEN. ♂ Solid color; black tongue and switch.
			No. 75273. RIOTERS PRETTY PEARL. ♀ Solid color; black tongue and switch.
		No. 152789. ♀ NORA OF MONMOUTH. Solid color, light tongue, black swtch.	No. 45313. ♂ LORD HARRY'S HUGO. Solid color; black tongue and switch.
			No. 34117. FLORIANS FANCY. ♂ Solid color; black tongue and switch.
			No. 83743. PRIDE OF PIONEER. ♀ Solid color except little white on belly; white tongue and black switch.
			No. 135194. ♀ PRIDE OF PIONEER 2D. Solid color, black tongue and switch.
			No. 34117. FLORIANS FANCY. ♂ Solid color; black tongue and switch.
			No. 83743. PRIDE OF PIONEER. ♀ Solid color except little white on belly; white tongue and black switch.

In consideration of the single-factor hypothesis for the different white areas the following animals are of especial interest: S. Boyle, D. Fontaine 4th, S. Fontaine's King, Rioter Pretty Belle, and Caiest.

The sire of the dam of the father of Lakeland's Poet is known to have been star. This would go to his father's dam as a single dose. The chance of Mabel's Poet 65780 carrying it would be even and of Lake-

land's Poet receiving it 1 in 4. The only pedigrees which we have been able to study have shown a fair chance for the animals to carry the star. There is in this pedigree an apparent exception to the recessive quality of star, as the mating of S. Boyle (star) by D. Fontaine 4th (star) gave a solid-color offspring.

Some interesting cases of the reappearance of the star after its disappearance in the F_1 generation may be mentioned. Taurus Creamelle Hengerveld mated to No. 12, the F_1 exception noted above, gave as the result of this back cross a star. Likewise, when Crossbred 0 (solid color carrying star) is inbred to the solid-color females carrying star, he gave two stars and one solid-color offspring. These F_2 offspring would indicate a segregation of the star determiner from solid color.

MARKINGS OF THE THROAT

In cattle the white on the neck may be divided into two classes, according to the center of its occurrence. These are (1) a white area on the throat and (2) an extension of the white belt of the withers into an irregular white area on the crest of the neck. This last marking is quite rare. In these experiments we have nothing which could be called this extension, and shall therefore have no opportunity to consider its behavior. The white spot on the throat as it appeared in the parents and in the offspring is given in Table XII.

TABLE XII.—*Behavior of the white markings on the throat in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Throat spot × solid color.	13 solid + 2 spot.
Do	Throat spot F_1 solid carrying white.	1 white.
Do	Throat spot × F_1 solid color.	1 solid color.
Kayan	Solid color × throat spot.	3 solid color.
Do	Solid color × solid color.	19 solid color.
Lakeland's Poet	Solid color × throat spot.	3 solid color.
Do	Solid color × solid color.	Do.
Minor crosses:		
Crossbred 0	F_1 solid carrying throat spot × solid color.	2 solid color.
Do	F_1 solid carrying throat spot × F_1 solid carrying throat spot.	1 throat spot × 2 solid color.
Crossbred 10	F_1 solid color × solid color.	1 solid color.
Lady Primrose's Governor of the Fountain.	Throat spot × solid color.	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Solid color × solid color.	Do.

The conclusion to be drawn from Table XII is that the white markings of the throat are recessive. There are two cases where the behavior of the F_1 offspring is doubtful: Crossbreds 6 and 20 from the same dam,

Maple Grove Netta, and by the same bull, Taurus Creamelle Hengerveld. Both animals have a throat spot similar to that of their sire. It would seem that Maple Grove Netta must also have this mark as a single dose. She is, however, given as unmarked by the herd book. Unfortunately, the Ayrshire herd books allow the record to be made as red and white without giving the actual location of these marks. We can not, therefore, trace definitely the white spot. The color pedigree of Maple Grove Netta is given to show the large amount of white in the pedigree and also the difficulty in tracing the throat mark (Table XIII).

TABLE XIII.—*Pedigree of Maple Grove Netta*

Maine Agr. Exp. Sta.—Standard Pedigree Blank

MAPLE GROVE NETTA. Sex ♀

No. 29307.

♂ No. 10034. BARCHESKIE COPESTONE. Brown and white.	No. "5665." ♂ BARCHESKIE MAY KING. Imported.	No. ♂	No. ♂
			No. ♀
		No. ♀	No. ♂
			No. ♀
	No. "11918." ♀ ORANGE BLOSSOM OF BARCHESKIE. Imported.	No. ♂	No. ♂
			No. ♀
		No. ♀	No. ♂
			No. ♀
♀ No. 15474. LENETTA. Dark red, with white spot on forehead; flanks white; white strip on forelegs; white switch.	No. 5389. ♂ GLOUCESTER. Dark red, with heart-shaped spot of white in forehead; small strip of white back of shoulders; two small white spots back of left shoulder; white on both flanks.	No. 4227. ♂ GEORGE A. F. Dark red and white.	No. 2469. GLENCARNE. ♂ Dark red; little white; white in forehead; white spot on each shoulder; off hip and both flanks white; spotted legs.
			No. 4464. QUEEN OF AVY 3D. ♀ Red and white; three-eighths white; some white in forehead inclosing red spot.
		No. 9358. ♀ MALINDA B. Dark red; white spot in forehead; white on flank and tail.	No. 2083. HEBRAN. ♂ Red and white.
			No. 6190. QUEEN LINDETTA. ♀ Dark red; a little white; white spot in forehead.
	No. 11224. ♀ ATHENIA. Dark red and white; forehead white with two narrow strips of red inclosed; spot of white above left nostril; smaller spot above right; strip across shoulders; both thighs white; band of white across base of tail.	No. 4213. ♂ LINWOOD. Dark red and white; white splash in forehead.	No. 3862. CLIMAX. ♂ Red and white.
			No. 6579. LADY TEAZLE. ♀ Reddish roan; V of white in forehead; white strip over loin and shoulders.
		No. 4542. ♀ MAID OF ATHENS. Red and white.	No. 1961. LORD DERBY. ♂ White and red; mostly white.
			No. 4548. STRATHAVEN MAID. ♀ Light red and white.

It is hard to do much with this pedigree in the way of tracing the inheritance of the separate white marks. The presence of a large number of them is shown as many areas carried by a single animal.

On the hypothesis suggested there are two cases of segregation: Taurus Creamelle Hengerveld with a white throat mated to No. 2 solid color carrying white throat gave No. 49, a white-throated offspring. Crossbred 0 when mated to Crossbred 1 both solid color carrying white throat recessively produced Crossbred 25, a white-throated heifer.

MARKINGS OF THE SHOULDERS

The markings occurring in the region of the shoulders may be divided into three categories, depending on the amount of extension of the band across the shoulders. The first of these is a band extending from under the armpits on one side across the withers to the armpits on the other side. The band may be broken in its lower part by pigment, so that all there is left is a band across the withers. This band may be asymmetrical, having a greater area on one side than the other. Unfortunately all of these extensions of this spot of pigment are not present in the herd. Attention will be confined to the presence or absence of the white mark. This treatment is given in Table XIV.

TABLE XIV.—*Behavior of the white markings on the shoulders in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Shoulder band × solid color.	1 spotted right shoulder, 2 shoulder bands, 11 solid color.
Do	Shoulder band × F_1 solid carrying band.	1 solid color.
Do	Shoulder band × F_1 shoulder band.	1 shoulder band.
Kayan	Solid color × spots on left shoulder.	2 solid color.
Do	Solid color × shoulder band.	10 solid color.
Do	Solid color × solid color	9 solid color.
Lakeland's Poet	Solid color × shoulder band.	1 solid color.
Do	Solid color × solid color	5 solid color.
Minor crosses:		
Crossbred 0	F_1 solid carrying shoulder band × solid.	2 solid color.
Do	F_1 solid carrying shoulder band × F_1 solid carrying shoulder band.	2 shoulder bands+1 solid color.
Crossbred 10	F_1 solid color × shoulder band.	1 solid color.
Lady Primrose's Governor of the Fountain.	Shoulder band × solid color.	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Solid color × solid color	Do.

The data of Table XIV would indicate the recessive character of the white markings of the shoulders. On the basis of this conclusion there are two cases of segregation: Crossbred 0 mated to heifers known to carry

this mark but not showing it somatically produced two offspring with shoulder bands and one without it.

There are three apparent exceptions to this recessive interpretation. Two bulls from Maple Grove Netta (No. 6 and 20) have shoulder bands. By the pedigree given in Table XIII it is shown that Maple Grove Netta must have been heterozygous for this mark; consequently it is not to be wondered at that her offspring have it when mated to a bull carrying it. The other case is not quite so easy to reconcile. In the pedigree given in Table XVI with this single-factor hypothesis there are two known possibilities in the fourth generation to have this factor transmitted to Flying Fox's Flora, the mother of the aberrant individual. This doe not leave a great chance for her to have this factor. From her other breeding, however, it would look as if Flying Fox's Flora must carry this white mark.

MARKINGS OF THE RUMP

The white markings of the rump consist chiefly of a spot whose center is the tail set. This spot may be extended by fusion with the patches having as their centers the middle of the flanks. These extensions will be considered as two separate spots located, respectively, on the rump and on the flanks. The behavior of the rump spot from the parents to the offspring is shown in Table XV.

TABLE XV.—*Behavior of the white markings on the rump in the parental and first filial generation*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Rump spot × rump spot	2 rump spots.
Do	Rump spot × solid color	10 solid color.
Do	Rump spot × F_1 solid carrying rump spot.	1 rump spot, 1 solid color.
Kayan	Solid color × rump spot	15 solid color.
Do	Solid color × solid color	7 solid color.
Lakeland's Poet	Solid color × rump spot	1 rump spot, 2 solid color.
Do	Solid color × solid color	3 solid color.
Minor crosses:		
Crossbred 9	F_1 solid color carrying rump spot × solid color.	2 solid color.
Do	F_1 solid color carrying rump spot × F_1 solid color carrying rump spot.	1 rump spot.
Crossbred 10	F_1 solid color carrying rump spot × rump spot.	1 solid color.
Lady Primrose's Governor of the Fountain.	Rump spot × solid color	Do.
Johanna Lad Manor De Kol.	do	1 rump spot.
Columbia's Fox	Solid color × solid color	1 solid color.

Here, also, the character of the white mark appears recessive in its transmission. There are, however, three exceptions to this view, if these should prove genetically what they appear to be somatically. The

exceptions are Crossbreds 11, 14, 35, and require for their production that the animals Lakeland's Poet and Flying Fox's Flora be heterozygous for the factor producing this white spot.

The single case of this white in the pedigree makes it hard to believe that Flying Fox's Flora carries this mark, even though her two offspring do show it somatically. The case of Lakeland's Poet is not quite so bad, as in his pedigree there are two animals, S. Fontaine's King and Rioter's Pretty Bell, which have their tails recorded as white. Since the tail set is the common center for the rump spot, it is probable that these animals have this spot (Table XVI).

TABLE XVI.—*Pedigree of Flying Fox's Flora*

Maine Agr. Exp. Sta.—Standard Pedigree Blank.

Sex ♀				
FLYING FOX'S FLORA.	♂	No. 61441. ♂ CHAMPION FLYING FOX. Solid color, except white on brisket; white tip to black tongue; black switch.	No. P. S. 2160. ♂ GOLDEN FERN'S LAD. White spot on forehead and withers; tongue and switch white.	No. 1559. BOYLE. ♂ Brown and white; white on forehead; tail and tongue white.
				No. 4711. GOLDEN FERN. ♀ Dark fawn; solid color; black tail.
			No. 149740. ♀ SULTANA'S ROSETTE. Solid color; black tongue and switch.	No. P. S. 797. SARABOND. ♂ Dark brown; two white specks on fore fetlocks; tail black and white.
				No. P. S. 2128. ROSETTE 4TH. ♀ Gray; white on right hip; tail black; dappled under barrel.
No. 274051.	No. 64768. FLYING FOX'S VICTOR. Solid color, black tongue and switch.	No. F. S. 8801. ♀ DULCE OF OAKLAND. Solid color; black tongue and switch.	No. ♂	No. ♂
				No. ♀
			No. ♀	No. ♂
				No. ♀
	♀	No. 42000. ♂ DON OF HOOD FARM. Solid color; black tongue and switch.	No. 30212. ♂ PEDRO SIGNAL LANDSEER. Solid color; black tongue and switch.	No. 9033. YOUNG PEDRO. ♂ Solid color; black tongue and switch.
				No. 60789. SIGNAL MINE 2D. ♀ Solid color; black tongue and switch.
			No. 82426. ♀ DONNEY POGIS 2D. Solid color; black tongue and switch.	No. 23015. TENNESSEE'S LANDSEER. ♂ Few white hairs on left shoulder; small white spot on right flank; white ankles, tongue, and switch.
				No. 38907. DONNEY POGIS. ♀ Solid color; black tongue and switch.
No. 176058.	BLONDDRUNA. Solid color, black tongue and switch.	No. 124651. ♀ BLONDE BRIGGS. Solid color; black tongue and switch.	No. 26930. ♂ GEM OF ST. LAMBERT. Solid color; black tongue and switch.	No. 18453. COLUMBINE'S JOHN BULL. ♂ Solid color; black tongue and switch.
				No. 48577. MISS CARO POGIS. ♀ Solid color; black tongue and switch.
			No. 67895. ♀ BANGOR MAID. Solid color, except white on belly, brisket; mixed switch; black tongue.	No. 21455. FAUNTLEROY. ♂ Some white on brisket; small white spot on each flank; white tongue; black switch.
				No. 53505. EUNA'S FIRST. ♀ Solid color, except white spot on each stiffle and flank; white tongue; black switch.

The white shoulder marks of Golden Fern's Lad and Tennessee's Landseer are of interest in connection with the inheritance of this white. The presence of white spots on the hips of Rosette 4th is of interest,

since this appears to be the only animal known to four generations which could transmit this white to Flying Fox's Flora. Tennessee's Landseer, Fauntleroy, and Euna's First have white marks on the flanks. In connection with the development of the separate factor argument for the individual white spot, the animals Bangor Maid, Boyle, and Sarabond are of interest.

MARKINGS OF THE FLANK

Markings on the flanks of dairy cattle generally consist rather of extension areas from the white on the legs than they do of definitely centered spots. However, from the evidence brought forward by Allen (*1*) it would appear that this is a definite area. It consequently will be treated as such. Table XVII gives such treatment of the parental and F_1 generation.

TABLE XVII.—*Behavior of the white markings on the flanks in the parental and first filial generation*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Spotted left flank × solid color.	3 spotted, 10 solid color.
Do	Spotted × spotted	1 solid color.
Do	Spotted × F_1 solid color carrying spotted.	1 spotted.
Do	Spotted × F_1 spotted	Do.
Kayan	Solid color × solid color	9 solid color.
Do	Solid color × spotted flanks.	13 solid color.
Lakeland's Poet	Solid color × spotted flanks.	2 solid, 1 left flank spotted.
Do	Solid color × solid color	3 solid color.
Minor crosses:		
Crossbred 0	F_1 solid color carrying spotted flanks × F_1 spotted flank.	2 spotted flanks.
Do	F_1 solid color carrying spotted flanks × solid.	2 solid color.
Do	F_1 solid color carrying spotted flanks × F_1 spotted flanks carrying solid color.	1 solid color.
Crossbred 10	F_1 solid color carrying spotted flanks × spotted flanks.	Do.
Lady Primrose's Governor of the Fountain.	Spotted flanks × solid color.	Do.
Johanna Lad Manor de Kol.	do	1 spotted right flank.
Columbia's Fox	Solid color × solid color	1 solid color.

Here, again, the white areas are generally suppressed in the offspring when an animal bearing them is crossed to a solid-color animal. There are six cases apparently at variance with this conclusion. Five of them may be easily seen by their pedigrees to have had an animal carrying the mark so close that either the aberrant parent would be heterozygous or else had an even chance for a single dose of the factor. Of the four

exceptions from Taurus Creamelle Hengerveld, two (Nos. 6 and 20) are from Maple Grove Netta. The pedigree of this animal given above shows that she must be heterozygous for this mark on a single-factor hypothesis. No. 14 by the same bull from Flying Fox's Flora is also an exception. In this case the parent expected to transmit this mark is in the second generation. The same is also true for the case of the two exceptions from Lakeland's Poet, for the parent carrying this mark is also in the second generation. It is interesting in this connection to compare the size, shape, and position of the flank markings of a daughter of Lakeland's Poet with the flank markings of her offspring from a straight F_2 generation. It will be noted that they are almost identical.

The remaining case is that of No. 3 from Johanna Lad Manor De Kol and Flora's Golden Poetess. The solid-color parent, Flora's Golden Poetess, has a white-flanked grandfather (Mabel's Poet) and granddam (Imp. Lady Grandiflora). It is not strange, then, that she should carry white for the flank.

MARKINGS OF THE TAIL

TABLE XVIII.—*Behavior of the white markings on the tail in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Lower half white × corner half white.	2 half white.
Do	Lower half white × solid color.	3 lower half white, 9 solid color.
Do	Lower half white × F_1 lower half white.	2 lower half white.
Kayan	Solid color × upper half white.	3 solid color.
Do	Solid color × lower half white.	11 solid color.
Do	Solid color × solid color	8 solid color.
Lakeland's Poet	Solid color × lower half white.	1 lower half white, 1 solid color.
Do	Solid color × solid color	3 solid color.
Minor crosses:		
Crossbred 0	Solid color carrying lower half white × solid color.	2 solid color.
Do	Solid color carrying lower half white × F_1 solid color carrying half white.	2 half white + 1 solid color.
Crossbred 10	Solid color × lower half white.	1 solid color.
Lady Primrose's Governor of the Fountain.	Lower half white × solid color.	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Solid color × solid color	Do.

The markings of the tail may be divided into four general groups: In the first only the switch is white; in the second the hair just above the switch is white; in the third group about half the tail is white; and in the fourth group the whole tail is white. Much observation of the switch and tail colors in cattle has led to the view that the color of the hair in

the switch is separate in its behavior from the rest of the tail. These areas will be studied separately. The cases of the ring just above the switch and of the whole tail white will not be studied, as these marks are not present in the herd. The choice of the broad quantitative group lies in a study of the length of those white areas. The length of the white area on the tail was measured and found to be between one-third and one-half the total length of the tail. The data have been considered on the basis of this and made two qualitative groupings: (1) half white and (2) solid color (Table XVIII).

TABLE XIX.—*Pedigree of Lassie of M. F.*

Maine Agr. Exp. Sta.—Standard Pedigree Blank.

Sex ♀				
LASSIE OF M. F.				
No. 297736.	♂ No. 92626. Pogis 95th of Hood Farm. Solid color, black tongue and switch.	No. 55552. ♂ Hood Farm Pogis 9th. Solid color, black tongue and switch.	No. 40684. ♂ Hood Farm Pogis. Solid color, black tongue and switch.	No. 17165. Oonan's Pogis. ♂ Solid color, black tongue and switch.
				No. 60738. Kathletta's Fancy. ♀ Solid color, black tongue and switch.
			No. 76106. ♀ Figgis. Brisket, armpits, belly, and hind stockings white; white on right foreankle and left foreleg; dark hairs in white switch.	No. 20883. Sophie's Tormentor. ♂ Star; brisket and left stifle white; white on flanks, belly, forefeet, and hind legs; white switch.
				No. 48326. Birdsey's Surprise. ♀ Belly and hind legs white; black and white switch; black tongue.
		No. 198780. ♀ Belle 7th of Hood Farm. Solid color, black tongue and switch.	No. 64268. ♂ Hood Farm Golden Lad. Solid color, black tongue and switch.	No. 57788. Financial King. ♂ Solid color, dark tongue and switch.
				No. 114438. Silent. ♀ Solid color, black tongue and switch.
			No. 180932. ♀ Tonoma 22d of Hood Farm. Solid color, black tongue and switch.	No. 25204. Torono. ♂ Star; white stifle; flanks, belly, right forearm, fore pasterns, and hind legs, half of tail and switch white.
				No. 147794. Oonan 14th of Hood Farm. ♀ Solid color, black tongue and switch.
	♀ No. 234891. Hood Farm Fern's Lassie. Solid color, black tongue and switch.	No. 80437. ♂ Hood Farm Golden Fern's Lad. Solid color, black tongue and switch.	No. 65300. ♂ Imp. Golden Fern's Lad. Star; white on stifles and right flank; streak on left flank/ little on belly and legs; white tongue and switch.	No. P. S. 1559. Boyle. ♂ Brown and white spot on forehead, tail, and white tongue.
				No. P. S. 4711. Golden Fern. ♀ Dark fawn; solid color; black tail.
			No. 195756. ♀ Scot's Belle. Solid color, black tongue and switch.	No. P. S. 2153. Great Scot. ♂ Gray-brown; solid color.
				No. P. S. 5431. Pallus. ♀ Gray; solid color; tongue and tail black.
		No. 212614. ♀ Belle 21st of Hood Farm.	No. 64268. ♂ Hood Farm Golden Lad. See above.	No. ♂
				No. ♀
			No. 184981. ♀ Hood Farm Pride. Solid color, black tongue and switch.	No. 56742. Oscar of Maplecroft. ♂ Solid color, black tail and tongue.
				No. 111172. Emma Hotchkiss. ♀ Solid color; white tongue; black switch.

The general conclusion from these data is that white on the tail is generally suppressed in the offspring when the animal bearing it is crossed with a solid-color animal. In this, also, there are some of the same irregularly behaving individuals which appeared in the previous

studies. Of the offspring of Taurus Creamelle Hengerveld, No. 14, 16, and 50 behave in contradiction to expectation. Of these the anomalous parents of No. 14 and 46 are available for study. The same difficulty of reconciling the behavior of No. 14 with expectation is experienced as in the preceding case. There are only three parents up to the fifth generation which carry the white.

It will be noted from the color pedigree of Lassie of M. F. that the chance of her carrying white for the legs is good, since two parents, Figgis and Imp. Golden Ferris Lad, both had these marks. The same is also true for markings on the tail (Table XIX).

The case of Lassie of M. F. is much better, as she had both of her grandfathers carrying the factor. The other exception is that of No. 11 by Lakeland's Poet. It will be noted in the previous pedigree that there is a good chance for this animal to carry the factor for a white tail, as the description indicates that this mark is carried by his father.

MARKINGS OF THE BELLY

TABLE XX.—*Behavior of the white markings on the belly in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	All white × inguinal spot.	2 all white + 1 solid color + 1 inguinal spot.
Do	All white × fore part white.	2 all white.
Do	All white × all white	1 all white.
Do	All white × solid color	5 all white + 1 solid color.
Do	All white × F_1 all white	2 all white.
Kayan	Inguinal spot × all white	7 inguinal spot, 2 belly white to navel, 2 belly all white, 3 solid color.
Do	Inguinal spot × solid color	4 inguinal spot, 4 solid color.
Lakeland's Poet	Solid color × inguinal spot	1 inguinal spot.
Do	Solid color × all white	3 all white.
Do	Solid color × solid color	2 solid color.
Minor crosses:		
Crossbred 0	Fore and hind part white carrying solid color × solid color.	Do.
Do	Fore and hind part white carrying solid color × all white.	1 all white + 1 solid color + 1 half white.
Crossbred 10	Spot in inguinal region × all white.	1 solid color.
Lady Primrose's Governor of the Fountain.	All white × solid color	Do.
Johanna Lad Manor De Kol	do	1 all white.
Columbia's Fox	Solid color × solid color	1 inguinal spot.

White on the belly takes a variety of patterns, depending largely on the method of extension of these areas. The primary area, if it may be spoken of as such, is a white spot in the region of the udder. This is practically always present when any of the other extensions toward

the brisket are present. Since the data on the extension areas are rather limited, it has been deemed well to consider only this inguinal spot and leave the extension factors for future study (Table XX).

It is obvious at a glance that this white on the belly behaves quite differently from the white markings on the rest of the body. The general conclusion to be drawn from a study of Table XX is that white in the region of the udder behaves as if it were a dominant. There is unfortunately one real exception to this, in that No. 13 has a white throat and inguinal spot, when his parents are both solid color. For the case it must be said that the description of the parent, Columbia's Fox, had to be taken from the herd book, as this animal was sold without description. It is quite probable that a small white spot would escape the one making the record for registry, so that when it is possible to check this case it may be that the required spots will be found.

Should the interpretation of a dominant for this case be correct, the expectation and realization for the various classes would be as follows:

Inguinal spot × inguinal spot = 3 spots to 1 solid color. The actual figures obtained are 21 to 6. Inguinal spot × solid color would be expected to give 1 to 1; the experimental results obtained are 10 to 8.

WHITE MARKINGS OF THE LEGS

The markings of the legs may be divided into four general classes: (1) Animals which are solid color; (2) animals with a white ring on the coronet and extending a short distance above it; (3) the further extension of this white into a white sock; and (4) its still further extension into a white stocking. There are other markings, but these are the most common and will be the only ones considered.

TABLE XXI.—*Behavior of the white markings on the legs in the parental and first filial generations*

LEFT FORELEG

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld	Short stocking×long stocking	2 long stockings.
Do	Short stocking×short stocking	1 short stocking.
Do	Short stocking×solid color	1 short, 10 solid color.
Do	Short stocking×F_1 long stocking	1 long stocking.
Do	Short stocking×F_1 short stocking	1 short stocking.
Kayan	Solid color×long stocking	10 solid color.
Do	Solid color×short stocking	2 solid color.
Do	Solid color×solid color	10 solid color.
Lakeland's Poet	do	3 solid color.
Do	Solid color×long stocking	2 solid color+1 long stocking.
Minor crosses;		
Crossbred 9	Solid color carrying white×solid color.	2 solid color.
Do	Solid color carrying white×solid color carrying white.	1 spot above knee+1 small white spot on coronet+1 short stocking.
Crossbred 10	Solid color carrying white×solid color.	1 solid color.
Lady Primrose's Governor of the Fountain.	Long stocking×solid color	Do.
Johanna Lad Manor De Kol	do	Do.
Columbia's Fox	Solid color×solid color	Do.

TABLE XXI.—*Behavior of the white markings on the legs in the parental and first filial generations*—Continued

RIGHT FORELEG

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld	Long stockings×long stockings	1 long stocking.
Do	Long stockings×short stockings	1 short stocking.
Do	Long stockings×solid color	10 solid color, 1 short stocking.
Do	Long stockings×F_1 long stockings	1 long stocking.
Do	Long stockings×F_1 short stockings	Do.
Kayan	Solid color×solid color	12 solid color.
Do	Solid color×short stockings	2 solid color.
Do	Solid color×long stockings	8 solid color.
Lakeland's Poet	Solid color×solid color	3 solid color.
Do	Solid color×long stockings	2 solid color, 1 long stocking.
Minor crosses;		
Crossbred o	Solid color carrying white stockings×solid color.	2 solid color.
Do	Solid color carrying white stockings×solid color carrying white stockings.	1 white stocking+1 white spot above knee+1 white on coronet.
Crossbred 10	Solid color carrying short stockings ×solid color.	1 solid color.
Lady Primrose's Governor of the Fountain.	White stockings×solid color	Do.
Johanna Lad Manor De Kol	Long white stockings×solid color	Do.
Columbia's Fox	Solid color×solid color	Do.

LEFT HINDLEG

Taurus Creamelle Hengerveld	Long white stocking×F_1 short stocking.	1 long white stocking.
Do	Long white stocking×F_1 long stocking.	Do.
Do	Long white stocking×long white	3 long white stockings.
Do	Long white stocking×solid color	7 solid color, 1 long white, 3 short.
Do	Long white stocking×short stocking.	1 short stocking.
Kayan	Solid color×long white stocking	12 solid color.
Do	Solid color×long stocking	10 solid color.
Lakeland's Poet	Solid color×solid color	3 solid color.
Do	Solid color×long stockings	1 spot on thigh, 2 solid color.
Minor crosses;		
Crossbred o	Solid color carrying white×solid color.	1 solid color.
Do	Solid color carrying white×solid color carrying white.	1 long stocking, 1 small white spot above coronet.
Crossbred 10	Solid color carrying white×solid color.	1 solid color.
Lady Primrose's Governor of the Fountain.	Long stockings×solid color	Do.
Johanna Lad Manor De Kol	....do	1 short stocking.
Columbia's Fox	Spot on thigh×solid color	1 solid color.

RIGHT HINDLEG

Taurus Creamelle Hengerveld	Long white stockings×long white stockings.	3 long white stockings.
Do	Long white stockings×solid color	4 short stockings, 2 long white stockings, 5 solid color.
Do	Long white stockings×F_1 long white stockings.	2 long white stockings.
Kayan	Solid color×solid color	10 solid color.
Do	Solid color×long white stockings	12 solid color.
Lakeland's Poet	Solid color×solid color	3 solid color.
Do	Solid color×long white stockings	2 solid color, 1 white spot on flank.
Minor crosses;		
Crossbred o	Solid solor carrying white stockings×solid color.	2 solid color.
Do	Solid color carrying white stockings×solid color carrying white stockings.	1 long white stocking+2 solid color.
Crossbred 10	Solid color carrying white stockings×solid color.	1 solid color.
Lady Primrose's Governor of the Fountain.	Long white stockings×solid color	Do.
Johanna Lad Manor De Kol	....do	Do.
Columbia's Fox	Solid color×solid color	Do.

From unpublished data collected by Dr. A. H. Sturtevant, of Columbia, and the writer on horses it was shown that the frequency of white on a given leg was markedly different. The order of appearance is left hind, right hind, left fore, and right fore. This conclusion agrees with what Brewer (*11*) had already published in 1882, unknown to us, as to the frequency of these marks. It has seemed, therefore, that in this study the individual leg markings should be treated separately (Table XXI).

TABLE XXII.—*Pedigree of Flora's Golden Poetess*

Maine Agr. Exp. Sta.—Standard Pedigree Blank.

FLORA'S GOLDEN POETESS. Sex ♀

No. 264927.

No. 81118. FONTAINES CAIEST. Solid color, black tongue and switch. ♂	No. 65780. ♂ MABEL'S POET. Some white on brisket; speck on left side of belly and patch on belly near left flank; black tongue and switch.	No. P. S. 2591. ♂ CAIEST. White under chest; fore legs, switch white.	No. 2207. S. FONTAINE'S KING. ♂ White on each stifle; tail and tongue white.
			No. 3582. D. OXFORD LASS. ♀ White across withers; tongue black; tail white.
		No. P. S. 6311. ♀ MABEL 35TH. Solid color, red muzzle.	No. 1559. S. BOYLE. ♂ White spot on forehead; tail and tongue white.
			No. 3213. D. MABEL 23D. ♀ Tongue and tail black; dappled on chine.
	No. 195762. ♀ FONTAINE'S BROWN DAISY. Solid color, black tongue and switch.	No. P. S. 2591. ♂ CAIEST. See above.	No. ♂
			No. ♀
		No. P. S. 6325. ♀ BROWNY'S BEAUTY. Tongue and switch black.	No. 1690. S. MARION. ♂ Solid color, black tongue and switch.
			No. F. S. 7623. D. BEAUTY BROWNY. ♀ Black points.
No. 219544. FLYING FOX'S GRANDIFLORA. Solid color, black tongue and switch. ♀	No. 64768. ♂ FLYING FOX'S VICTOR. Solid color, black tongue and switch.	No. 61441. ♂ CHAMPION FLYING FOX. Solid color, except white on brisket; white tip to black tongue; black switch.	No. P. S. 2160. GOLDEN FERN'S LAD. ♂ White spot on forehead and withers; tongue and switch black.
			No. 149740. SULTANA'S ROSETTE. ♀ Solid color, black tongue and switch.
		No. F. S. 8801. ♀ DULCE OF OAKLAND. Solid color, black tongue and switch.	No. ♂
			No. ♀
	No. 173908. ♀ IMP. LADY GRANDIFLORA. Spot on left hip; white on right stifle and hind fetlocks; black tongue and switch.	No. 52915. ♂ MONA'S GLORY. Solid color, black tongue and switch.	No. P. S. 1242. GOLDEN LAD. ♂ Solid color, tongue and switch black.
			No. P. S. 3983. MONA 8TH. ♀ Spots in forehead; tail white; white patch on each stifle.
		No. 165772. ♀ LADY GRANDIFLORA. Solid color, black tongue and switch.	No. 1242. GOLDEN LAD. ♂ Solid color, black tongue and switch.
			No. F. S. 6407. GRANDIFLORA. ♀ Solid color, tail and tongue black.

The general conclusion is that these white areas are generally suppressed in the offspring when the animal bearing them is crossed to a solid-color animal. Should this be interpreted as being due to a single recessive factor, several of the experimental animals have white markings against this interpretation. Two of these animals are anomolous to this in every foot. These are Crossbreds 11 and 35. Study of the pedigree of Lakeland's Poet, the sire of these animals, shows that he had a good

chance to carry this white, as his mother, Nora of Monmouth II, had white ankles and a white knee, and his grandfather, Caiest, had four white feet. The case of Flying Fox's Flora is not so clear. The only ancestor in the four generations of her pedigree who carried white feet is Tennessee's Landseer. The chances of her carrying factors for the production of these feet derived from this animal are small, as the animal bearing such marks is in the fourth generation.

It will be noted that the hind feet have more exceptions than the fore feet. Three of these females exceptional in the left hind foot are also exceptions in the right hind foot. These three exceptions are Lassie of M. F., Eventime 4th, and Hearthbloom. Only one of these animals, Lassie of M. F., is available for study. The chance of her carrying white is rather good, as she has two ancestors in the third generation who could transmit this to her. The other exceptions are Flora's Golden Poetess, with offspring having white on the left hind feet; Columbia's Brown Bessie, and Orono Ellen, with offspring with white on the right hind feet. The chance of Flora's Golden Poetess carrying the white seems good from the above pedigree. Caiest in the third generation and Imp. Lady Grandiflora both carry white. This makes it certain that the mother and the two grandparents on the father's side each has one dose of the factor (Table XXII). The case is even stronger for Columbia's Brown Bessie, as in her ancestry both of her grandmothers have white feet. In fact, the wonder is why she did not transmit more white to her offspring.

Summary of the Evidence on the Separate Inheritance of White Markings

A general summary of the behavior of the white markings just studied seems necessary for a clear understanding of the conclusions based on this study and their bearing on the general problem of coat-color inheritance. As has been previously pointed out, the limiting of the study to individual spots attacks the problem in an entirely novel way. Such analysis is made necessary because of the peculiar ratios which have been obtained in other studies of coat color, such as those made in the Shorthorns. In the study of the roan coat of this breed about the only thing which the results of Wilson (*49–56*), Laughlin (*19*), Wentworth (*44, 45*), Barrington and Pearson (*6*), and Walther (*43*) have in common, are exceptions which each found to the interpretations offered by the other writers. A beginning at a solution of these exceptions has been made by the excellent review of the writings of Storer, Wilsdorf, and others on white body color by Lloyd-Jones and Evvard (*20*). In this review they show that two types of identical white body with colored ears exist. In the Chillingham cattle this white is dominant. In the Highland cattle it is recessive.

This does not quite fit the case of the roan Shorthorn, for, while the presence of these two genetically different whites would complicate the

results, it is entirely likely that their presence would be noted because the pattern of each is so striking. It does remain to be shown rather that the piebald cattle, like the Shorthorn, have a difference in behavior of the separate spots which compose this piebald. A beginning at this kind of analysis has been made by Kiesel, according to a review by Lang (*18*). In these experiments a solid-color Limburger race was crossed to a piebald race, F_1 intermediate piebald. The back cross gave 22 solid color and 29 piebald. The back cross to the piebald gave 84 piebald out of 90. Unfortunately, no record of the exact spotting has been given; consequently, we are left in the dark concerning any difference in behavior throughout the coat. It would seem, however, that Kiesel's results would fall in line with the results obtained here, where each individual area is treated separately.

Analysis by the method of individual white spots has shown a marked difference in the inheritance of such spots throughout the animal's coat. This is perhaps as far as the writer should go, and is the only conclusion it is intended to emphasize; but realizing that there are exceptions not yet accounted for, it may be said that white spotting in the inguinal region is, broadly speaking, dominant. The spots of the rest of the piebald pattern are, individually considered, recessive. These enumerated individually according to the region in which they occur are (1) white on the face (star, star snip, or blaze); (2) white on the throat; (3) white as a band across the shoulders; (4) a white area on the rump at the base of the tail set; (5) white on the flanks as irregular spots; (6) white on the tail above the switch; and (7) the white stockings on the four feet.

The bearing of this difference in inherited behavior on the general problem is at once evident. If the red-coated Shorthorns should carry one of these recessive white spots, we should expect a small proportion of cattle produced from the random mating of such an animal in the Shorthorn population which would be white-spotted. In point of fact, this is what has actually been obtained. The reverse is also true that if these dominant white are mated together, we should expect that a heterozygous mating would now and then take place, giving a red. The evidence brought forward offers a straightforward, clear explanation of the anomolous behavior of the Shorthorn coat.

Behavior of the Muzzle Color in the F_1 Progeny

The categories used to describe the color of the muzzle pigment are, in general, the same as those used for the tongue color. The tabulation and classification of these terms has already been given by Pearl (*33*). For this classification the reader is referred to his paper. In his study of tongue color it was found that the essential thing was not so much the color of the pigment but rather its presence. This study will, therefore, be limited to the presence or absence of pigment. Table XXIII gives the tabulated data for this treatment.

TABLE XXIII.—*Behavior of the muzzle pigment in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	White×pigmented	13 pigmented.
Do	White × F_1, pigmented carrying white.	1 white with small black spots.
Kayan	Pigmented×pigmented	13 pigmented.
Do	Pigmented×white	3 pigmented.
Do	Pigmented×pigmented and white	Do.
Do	Pigmented×white, few pigmented spots.	1 pigmented.
Lakeland's Poet	Pigmented×pigmented	5 pigmented.
Do	Pigmented×pigmented, spotted	1 pigmented.
Minor crosses:		
Crossbred 0	F_1 pigmented carrying white×pigmented.	2 pigmented.
Do	F_1 pigmented carrying white×pigmented carrying white.	3 pigmented.
Crossbred 10	Pigmented carrying white×white, few black spots.	1 smoky brown.
Lady Primrose's Governor of the Fountain.	White×pigmented	1 pigmented.

The conclusion to be drawn from Table XXIII is that the pigmented muzzle is dominant to the nonpigmented one.

BEHAVIOR OF THE TONGUE PIGMENT IN THE F_1 OFFSPRING

The inheritance of tongue color in cattle has already been studied in this laboratory. The previous study on several thousand cattle indicates that pigmentation is due to two closely coupled factors. The data here are not sufficient to test this hypothesis thoroughly. As far as it goes, Table XXIV substantiates the previous conclusions.

TABLE XXIV.—*Behavior of the tongue pigment in the parental and first filial generation*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	White×pigmented	6 pigmented, 1 black and white.
Do	White×white	1 white.
Do	White×F_1 white	2 white.
Kayan	Pigmented×pigmented	6 pigmented.
Do	Pigmented×white	10 pigmented, 3 black and white.
Lakeland's Poet	Pigmented×pigmented	3 pigmented.
Do	Pigmented×white	2 white, 1 pigmented.
Minor crosses:		
Crossbred 0	Pigmented carrying white × pigmented.	2 pigmented.
Do	Pigmented carrying white × pigmented carrying white.	2 white.
Do	Pigmented carrying white×white	1 white.
Crossbred 10	F_1 pigmented carrying white×white	Do.
Lady Primrose's Governor of the Fountain.	White×pigmented	1 pigmented.
Columbia's Fox	Pigmented×pigmented	Do.

Pigmented is dominant to unpigmented tongue. Lakeland's Poet seems to contradict this but a study of his pedigree given above shows him heterozygous for the unpigmented tongue.

Inheritance of Switch Color

All of the crosses available for study in this experiment have either one or both of the parents with a black switch. It is impossible, therefore, to determine the dominance of the other colors to each other. Black as a color is dominant to the other colors (Table XXV).

Table XXV.—*Behavior of the switch color in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	White×black	1 black, 2 white, 2 black and white.
Do	White×white	2 white, 1 white with few black hairs.
Do	White×black, few gray hairs.	2 white, 1 white tip and 1 black.
Do	White×F_1 white	2 white.
Kayan	Black, few white hairs×black.	5 black.
Do	Black, few white hairs×white.	10 black, 1 black×white.
Do	Black, few white hairs×mixed red, black, gray.	3 black.
Lakeland's Poet	Black×white	1 black, 1 black×white, 1 black, few white hairs.
Do	Black×black, few white hairs.	3 black.
Minor crosses:		
Crossbred o	Black carrying white×black.	2 black.
Do	Black carrying white×black carrying white.	1 white.
Do	Black carrying white×black and white.	Do.
Do	Black carrying white×white.	1 black.
Crossbred 10	....do	1 red.
Lady Primrose's Governor of the Fountain.	White×black, few white hairs.	1 black.
Johanna Lad Manor De Kol	White×brown	1 black and white.
Columbia's Fox	Black×black, few white hairs.	1 black.

The apparent exceptions to this conclusion are shown by their pedigrees and their other breeding records to be heterozygous for the white factor.

Two interesting cases of segregation appear in the F_2 generation. A white switch resulted from mating Crossbred o (black carrying white) to an F_2 cow (black carrying white). Another case of segregation of perhaps more interest than the above is that from Crossbred 10 (black carrying white × white). This mating gave an orange-red—that is, the ground color of the Guernsey minus its diluting factor has been substituted for the white of the parent.

SEGREGATION OF THE POLLED CHARACTER

In his interesting review of the literature on domestic cattle and their origin Morse (*25*) and others (*5*) give briefly the theories as to hornless cattle (*47*). The different views held all go back to the conception of use in selection to account for the loss of horns. Durst (*14*) and Ewart (*15*) take the ground that domestication has brought about the loss of horns.

Auld (*4*) considers that a reduction in the horns took place in between the upper Eocene and lower Miocene period. Contradictory to this, Arenander (*2*) says the first cattle were hornless. Major (*23*) found skulls in the tertiary deposits in Italy the males of which were horned and the females hornless. Thus he considers a progressive extension of this would bring about the hornless race. This is something of the idea of Keller (*16*), when he cites the African cattle with movable horns to be ancestors of the hornless animals.

Based on rough notes collected at the Smithfield Club, Bateson and Saunders (*7*, *8*) treated these data on the horned character in cattle as if it were a single Mendelian factor and conclude that the presence and absence of horns are almost certainly allelomorphic characters. Further data collected by Spillman (*41*) on 165 cases led him to conclude that polledness is a simple Mendelian dominant. Boyd (*10*) has given later data on polledness in his cross of mutant polled Herefords onto pure horned Herefords and in his crosses of polled cows to the American bison. In both of these the polled character is dominant. In one of his bulls, Variation, he thinks he gets a significant difference from the expected ratio of 1 to 1. Thus, in crosses of Variation with pure horned cows he obtains 22 polled to 6 horned, or a difference from the expected 14 to 14 of 3.1 times the probable error of the theoretical half, or what might seem a significant difference. Lloyd-Jones and Evvard (*20*) have added materially to the data already presented. In 71 matings of Shorthorn bulls to Galloway cows 70 were clear-polled, 6 scurred, and 2 horned. This would seem to indicate that the polled factor is dominant. By this view the two horned heifers are exceptions to this conclusion. On this point, however, it seems well to quote them, as it appears to the author that the explanation they offer is correct. In reference to the dams of these horned heifers, they say (*20*, *p. 100a*):

> The cows 154 and 49 are referred to as "pure-bred Galloway," but their behavior in respect to the transmission of horns is not in harmony with what we should be justified in expecting if this were the case. Of all the polled breeds of cattle the Galloways have been longest established and, in the matter of horns perhaps the most rigidly selected, and they are recognized as practically never producing horned offspring. On the other hand, it must be pointed out that there does not exist an inevitable incompatibility between the heterozygous condition for polled, on the one hand, and the "pure-bred" condition, on the other. To be pure bred, from the breeders' standpoint, an animal must be recorded in the record books of the breed associations, or eligible for such record, or it must be from parents whose recent ancestors were thus recorded or eligible to record. To be sure, horned Galloways are not eligible to record, but the herd-book associations make no biological restrictions as to the

animal carrying horns recessive, and polled Galloway cows may not be disqualified as pure-breds because they happen to produce horned offspring. Therefore, although the behavior of the Galloway cows Nos. 154 and 49, one-half of whose offspring by a P_p bull have been horned, is "not in harmony with what we would expect" if they were pure-bred animals, nevertheless, it does not serve as conclusive evidence that they are not such. But if these two cows were *bona fide* pure-bred Galloways it is at least plain that we can not rely upon pedigree and registration as assurance that animals, purchased as pure-breds, are homozygous for the polled trait.

Thus, in the opinion of these authors the occurrence of horns on the heifers were due to their female parents being heterozygous for the horned factor.

In this same paper data on the segregation of polledness are given. In back crosses of P′p×pp (where P′ is the polled and p the horned factor) 7 polled to 9 horned were obtained where the expectation was 8 to 8. In P′p×P′p matings 20 polled to 8 horned were obtained where the expectation was 21 to 7. Their conclusion (*20, p. 102a*) on this evidence would seem substantiated:

> The present results substantiate the allelomorphic nature of the horned and polled condition in cattle.

The citations seem to prove conclusively that polledness is a simple Mendelian character. Some exceptions to this conclusion in our crosses, the results of which are presented in Table XXVI, gave ground for the belief that other factors influenced the result.

TABLE XXVI.—*Behavior of the polled character in the parental and first filial generations*

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Horns × horns	10 horns.
Do	Horns × polled	2 polled (1 ♀, 1 ♂), 2 loose scurs (1 ♀, 1 ♂).
Do	Horns × F_1 horns	1 horned.
Kayan	Polled × horns	8 polled (7 ♀, 1 ♂, slight prominence not through skin). 6 loose scurs (6 ♂). 3 tight scurs (3 ♂). 1 heavy horn (1 ♂).
Lakeland's Poet	Horns × horns	3 horns.
Do	Horns × polled	2 polled (2 ♀), 1 horned (♂).
Minor crosses:		
Crossbred 0	Horns × horns	5 horns.
Crossbred 10	Polled carrying horns × horns.	1 horned.
Johanna Lad Manor De Kol	Horns × horns	Do.

On the above interpretation, the data in Table XXVI offer several interesting anomalies. The first is an animal resulting from a mating of Kayan to the Ayrshire cow Dot Alaska. This mating produced a male, No. 21, with heavy 4-inch solidly attached horns (Pl. 6, B). By his previous breeding record, where each mating constituted a back cross,

it would seem that Kayan has been shown homozygous for the polled character, for if Kayan is considered heterozygous for the polled character, we have the impossible ratio of 17 polled to 1 horned, where the expectation is equality. Another assumption is left open: The scurred animals are really homozygous for the horned factor. This hypothesis seems unlikely on the following grounds: Scurs are present only where polled individuals are also in the stock. The inclusion of the scurred animals with the polled ones always improves back-cross ratios of polled × horned crosses, whereas the inclusion of scurred individuals with the horned group makes the ratios far from probable.

The same kind of case occurs in No. 31 out of the Aberdeen-Angus cow Eventime 4th. Since it has not been possible to test her genetic composition in so thorough a manner as that of Kayan, the case may be due to Eventime 4th being heterozygous for the horned factor.

Thus, we have two exceptional cases for polledness which demand a supplementary hypothesis to explain their appearance.

Spillman (*41*) says that scurs may develop to considerable size, but are loose and hollow. It is interesting in this connection, however, to point out that 8 loose- to 3 tight-scurred animals occur, a ratio corresponding well with the expectation of the F_2 population for a separate factor for loose and tight scurs. From this the most probable explanation would seem to be that loose and tight scurs are due to a simple Mendelian factor and not due to any inherent quality of being scurs.

In another paragraph in the same paper Spillman says that males are more likely than females to have scurs. This statement, however, is not supported by evidence, and in fact is contradicted by Lloyd-Jones and Evvard (*20*), who say their data—

> give no evidence that sex is in any way connected with the inheritance of these characters [Horned and polledness].

These investigators offer numerical data on the frequency of scurs. In 78 cases 6 were scurred and 2 horned. It will be seen that this frequency is much below that of the data of the author. Unfortunately Lloyd-Jones and Evvard do not sex their data; but it is possible to group the data of the present author in relation to sex, as is seen in Table XXVII.

TABLE XXVII.—*Relation of sex to polledness* [a]

Description of mating.		Polled.		Scurred.				Horned.	
Sire.	Dam.	Male.	Female.	Solid attachment.		Loose attachment.		Male.	Female.
				Male.	Female.	Male.	Female.		
Horned.....	Polled.....	1	3	.....	.....	1	1	1	.....
Polled.....	Horned.....	[b] 1?	7	3	.....	6	.....	1	.....

[a] This table is based on descriptions made before any idea of the influence of testicular secretion on the production of horns in the heterozygous individual was thought of.

[b] This male is recorded with loose scurs just under skin. He died and was put away before this could be checked.

The polled character in these crosses occurs most frequently in the females. In 7 offspring from matings of horned males with polled females, 3 polled females were produced to 1 polled male; 1 male and 1 female had scurs and 1 male had heavy horns. In the reciprocal cross of polled male bred with horned female, 1 male was doubtfully recorded as "loose scurs under the skin." He died before this could be checked. Of the others, 7 females were polled, 3 males had solidly attached scurs, 6 had loose scurs, and 1 was horned.

These data make it probable that sex has some influence on the horned condition. The parallel with the case of sheep is of special interest, for castration experiments by Wood and others (*57*) have established the presence of a secretion by the testis which materially aids the production of horns in this species. On the basis of this the testis in the bull would be expected to secrete a hormone which would produce horns with one dose of the horned genes, where two doses of the horned gene would be required by the female.

The parallel is still further emphasized by the variability, both intra- and inter-racially of the action of this secretion or hormone. Thus, Crossbred 9 (Pl. 4, A) at three years had scurs only, whereas Crossbred 21 at one year had heavy horns. For the case of sheep the work of Arkell and Davenport (*3*) have shown a similar length of time necessary for the action of the secretion in this species (*see also 13*).

This variability in the action may be the explanation of the results obtained by Lloyd-Jones and Evvard (*20*), where, out of 78 offspring of a Shorthorn bull to Galloway cows, they obtained only 6 scurred and 2 horned animals (see p. 45). Here it is conceivable that in this cross the secretion may be small in amount or lacking as in some of the merinos that Arkell bred. Such a lowered concentration or amount of the secretion would explain the results, as without its aid to the growth of horns the male offspring would be polled like the female, and the results obtained by Lloyd-Jones and Evvard would be expected. This difference in the behavior within a species is still further emphasized by the work of Morgan (*24*) on the hen-feathered races of poultry. In this case the hen-feathered character is known to vary all the way between a strictly hen-feathered male to one of almost complete cock plumage. This variability may also take place in the same bird of the Campine race owing to age or to a difference in the physiological state of the bird.

The position of cattle in the series of animals known to possess such a secretion seems intermediate between that of reindeer and sheep, since castration experiments on the horned breeds show no retarding of the horn growth, although it does tend to make the horn longer and more slender.

ON THE BEEF QUALITIES OF THE F_1 OFFSPRING

The qualities of an animal which make for the beef or dairy form seem to be divisible into four general regions when considered from the hereditary point of view. These regions from front to back are head, fore quarters, barrel, and hind quarters. It seems wise to have fairly broad categories under which to group the qualities of beef or milk production. In view of this the descriptive terms chosen to describe these animals are beef, beef and milk, milk and beef, and milk. Tables XXVIII shows the characters so grouped.

TABLE XXVIII.—*Behavior of the type of head, fore and hind quarters, and barrel, in the parental and first filial generation*

HEAD

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Milk × beef	3 beef and milk, 1 milk and beef.
Do	Milk × milk	10 milk.
Kayan	Beef × milk	4 beef, 14 beef and milk, 2 milk and beef.
Lakeland's Poet	Milk × beef	2 beef and milk, 1 milk and beef.
Do	Milk × milk	1 milk and beef, 2 milk.
Minor crosses:		
Crossbred o	do	5 milk.
Crossbred 10	Beef and milk × milk	1 milk and beef.
Lady Primrose's Governor of the Fountain.	Milk × beef	Do.
Johanna Lad Manor De Kol	Milk × milk	1 milk.
Columbia's Fox	Milk × beef	1 beef and milk.

FORE QUARTERS

Taurus Creamelle Hengerveld.	Milk × beef	3 beef and milk, 1 milk and beef.
Do	Milk × milk and beef	1 milk and beef.
Do	Milk × milk	8 milk.
Kayan	Beef × milk	6 beef, 14 beef and milk.
Lakeland's Poet	Milk × beef	3 beef and milk.
Do	Milk × milk and beef	2 milk and beef, 1 milk.
Minor crosses:		
Crossbred o	Milk × milk	5 milk.
Crossbred 10	Beef and milk × milk	1 milk and beef.
Lady Primrose's Governor of the Fountain.	Milk × beef	1 beef and milk.
Johanna Lad Manor De Kol	Milk × milk	1 milk.
Columbia's Fox	Milk × beef	1 beef and milk.

TABLE XXVIII.—*Behavior of the type of head, fore and hind quarters, and barrel, in the parental and first filial generation*

BARREL

Sire.	Description of mating.	Offspring.
Taurus Creamelle Hengerveld.	Milk × beef	4 milk and beef.
Do	Milk × milk and beef	1 milk.
Do	Milk × milk	6 milk.
Kayan	Beef × milk and beef	1 beef and milk, 1 milk and beef.
Do	Beef × milk	1 beef, 5 beef and milk, 11 milk and beef.
Lakeland's Poet	Milk × beef	3 milk and beef.
Do	Milk × milk and beef	1 milk.
Do	Milk × milk	1 milk and beef, 1 milk.
Minor crosses:		
Crossbred o	do	5 milk.
Crossbred 10	Beef and milk × milk	1 milk.
Lady Primrose's Governor of the Fountain.	Milk × beef	1 milk and beef.
Johanna Lad Manor De Kol.	Milk × milk	1 milk.
Columbia's Fox	Milk × beef	1 beef and milk.

HIND QUARTERS

Taurus Creamelle Hengerveld.	Milk × beef	4 milk and beef.
Do	Milk × milk and beef	1 milk.
Do	Milk × milk	8 milk.
Kayan	Beef × milk and beef	1 beef and milk, 1 milk and beef.
Do	Beef × milk	1 beef, 6 beef and milk, 11 milk and beef.
Lakeland's Poet	Milk × beef	3 milk and beef.
Do	Milk × milk and beef	1 milk and beef, 2 milk.
Minor crosses:		
Crossbred o	Milk × milk	5 milk.
Crossbred 10	$\frac{\text{Beef}}{\text{Milk}}$ × milk	1 milk.
Lady Primrose's Governor of the Fountain.	Milk × beef	1 milk and beef.
Johanna Lad Manor De Kol.	Milk × milk	1 milk.
Columbia's Fox	Milk × beef	1 beef and milk.

The dairy and beef qualities are seen to be blended to a considerable extent in the F_1 offspring. Even with this blending, however, dominance and recessiveness may be discerned in the crosses. The beef qualities are, in general, quite pronounced in the head and fore quarters of these hydrids. The dairy qualities seem to predominate in the barrel and hind quarters. This conclusion is further supported by a study of some exact measurements taken in various parts of the body of these animals. While the number of cows having these measurements is relatively small, they do show several interesting points. Taken without regard for their probable errors, the head of crossbreds with Angus blood is somewhat shorter in length and broader between the eyes than crossbreds of the dairy breeds of the same age. The girth at the last

rib for these Angus crossbreds is increased, as is also the width of the brisket. The measurements of the hind parts of the body show there is no change from the general form of the other crossbreds other than a slight shortening of the rump.

This conclusion is not in entire agreement with the few known recorded crosses where descriptions are given. Bruce (*12*) records crosses resulting from Dexter cattle with Shorthorns as wonderful beef animals. Boyd (*10*) says of his wide crosses of bisons with domestic cattle that the following characters are dominant: A somewhat modified hump of the bison, width of hind quarter, and width in front of the beef breeds. Nabours (*26*), describing the crosses made by Borden (*9*) of *Bos indicus* on *Bos taurus* says a modified hump, great increase in body size, and dewlap are characteristic of the F_1 progeny. The heavy filling in front is characteristic for all of these crosses. Bruce and Boyd, however, record an increase in the size and quality of the hind quarters. The author finds no such increase in his crosses.

From his results the author may say that for the improvement of the beef qualities of dairy breeds the first-generation crosses result in an increased value of the beef qualities in the fore quarters without materially influencing the hind quarters.

MILKING QUALITY OF THE F_1 OFFSPRING

Since the results on the milking qualities of the F_1 offspring are of interest, as they are new, it seems well to add them to this study, although they are as yet few in number. Tables XXIX and XXX give the age, days in milk, production, and the production expected from these animals when they reach their maximum at mature form. By forming a column for the difference in excess of the parent over that of the offspring it is possible to compare their productions and clearly bring out the differences. This same method may also be used for both fat and fat percentage. Tables XXIX and XXX give the results of such treatment. The quantity and the quality of the milk are separately treated, as it has been shown by Wilson (*49–56*), Pearson (*39*), and others (*40*), that the quality bears little relation to the quantity of the milk flow.

TABLE XXIX.—*Transmission of milk production from parental to first filial generation*

	Production of daughter.				Production of dam.				Difference daughter-dam production.
Mating.	Age.	Days in milk.	Production.	Correct maximum for 100 days.	Age.	Days in milk.	Production.	Correct maximum for 100 days.	
	Yr. m. d.		*Pounds.*	*Pounds.*	*Yr. m. d.*		*Pounds.*	*Pounds.*	*Pounds.*
101.....	2 0 5	110	2,016	2,666	10 8 14	104	3,579	3,600	— 934
102.....	2 7 26	105	3,035	3,849	3 9 5	96	2,243	2,846	+1,004
111.....	2 3 22	110	2,234	2,791	5 4 28	123	3,168	2,086	+ 105
112.....	2 4 7	93	2,312	3,405	4 4 5	109	1,830	1,881	+1,524

TABLE XXX.—*Transmission of fat concentration from parental to first filial generation*

Mating.	Production of daughter.				Production of dam.				Difference daughter-dam production.
	Age.	Days in milk.	Fat.	Correct maximum for 100 days.	Age.	Days in milk.	Fat.	Correct maximum for 100 days.	
	Yr. m. d.		*Per cent.*	*Per cent.*	*Yr. m. d.*		*Per cent.*	*Per cent.*	*Per cent.*
101.....	2 0 5	110	4.4	494	10 8 14	104	2.5	2.7	+1.7
102.....	2 7 26	105	3.2	392	3 9 5	96	3.4	3.5	− .3
111.....	2 3 22	110	3.2	3.2	5 4 28	123	3.6	3.8	− .6
112.....	2 4 7	93	3.5	3.5	4 4 5	109	5.4	5.6	−2.1

Tables XXIX and XXX show that high milk production tends to behave as dominant—that is, in a cross of high-producing lines to low-producing lines the offspring tends to have the high production of the high line. This agrees well with some unpublished studies on a large series where the productions of both parental lines are known.

Unfortunately a like result can not be said for percentage of fat. Here the parental high fat percentage is suppressed in the offspring when this parent is crossed to a low line.

SUMMARY

This constitutes a preliminary paper on the crossbred herd now being brought together by the Maine Agricultural Experiment Station for the purpose of studying some of the outstanding problems of dairy husbandry.

The first section of the paper is devoted to a study of the inbreeding in the foundation herd. It is shown that the inbreeding as measured by the best mathematical methods is no greater than would be expected to occur in any of the modern breeds when the animals were selected at random. Consequently it is safe to assume that the results of the study are not due to the width of the crosses, for, as has been pointed out, a number of the animals famous in their breed have been far more inbred than any of the parental stock used in these experiments.

The individual records of the animals composing both the parental generation and the first and second filial generations are given.

(1) Black body color is dominant to the other color in the first generation. In the second generation an orange-coated bull and a dark Jersey dun-coated heifer were segregated out. This is to be explained on the basis of a recessive dilutor in the Guernsey, segregated out along with the black color. The dark heifer shows that the Jersey does not normally possess this factor.

(2) It has been shown that white marking of the body taken as a whole appears as a dominant. Study of the individual white areas, however, indicate that this is due to white in the inguinal region only, for this

alone appears as such a dominant. The white spots on the face (star, star snip, and blaze), neck, shoulders, rump, flanks, and legs are, in general, suppressed in their offspring when such animals are mated to solid color.

(3) As has been suggested, but as has never been tested before, the pigmented muzzle is dominant to the one not so pigmented.

(4) Agreeing with the previous work of this laboratory it is shown that a pigmented tongue is dominant to a nonpigmented one.

(5) A black switch appears to cause the suppression of the other switch colors in the offspring. Because of this suppression and because all of the matings had at least one animal with a black switch as parent, it was impossible to study the behavior of the other colors. There was one case of segregation of a deep red-orange switch from a back cross of a black animal carrying an orange coat and white switch, genetically. This case showed the segregation of the factor for orange switch from that for both white and black.

(6) The character of polledness has been studied. Two-horned animals resulting from crosses of polled × horned appeared. On the basis of the other results these could have not resulted from a heterozygous polled condition. One of these cases had the horns tight on the head and the other loose. These cases then form exceptions to the previously accepted hypothesis of simple dominance for the polled character and require a subsidiary hypothesis. The hypothesis suggested is that the testes have some action on the presence or absence of horns. Partial proof to this hypothesis is given by the fact that of the polled animals 10 were females, 2 males, 1 doubtfully polled. Of those with scurs 1 female and 7 males had loose scurs; of those with tight scurs all (3) were males; of those with horns all (2) were males. This would seem like a clear case where the male has some influence. The explanation of this difference appears to be due to a hormone secreted by the germ cells. Should this prove true, this forms an interesting parallel between cattle and sheep, in which the sex glands are known to produce such changes.

(7) The qualities of beef production are shown to be divisible into four general regions of the body: head, fore quarters, barrel, and hind quarters. The type of head and heavy, deep fleshed fore quarters are transmitted to the offspring when either parent is of Aberdeen-Angus breed. The body and hind quarters appear intermediate, but resemble most the dairy parents.

(8) Data are given on the milk and fat production of some of the crossbreds. The results indicate that milk and fat production behave separately. High milk production is dominant to low, but high fat percentage is recessive to a low fat percentage in the milk.

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PLATE 1

A.—Eventime 4th: This is a good Aberdeen-Angus cow of rather light fleshing. Note the size of the udder as compared with that of Hearthbloom. This cow produced 2,852 pounds of milk for the year, while Hearthbloom produced only 500.

B.—Hearthbloom: The rounded blocky conformation is typical of the Aberdeen-Angus breed. Notice the cleanly polled condition. This animal is of better beef type than Eventime 4th.

C.—Orono Netta: Note the typical horns thrown up well over the head. The large amount of white distributed over the coat between the red or brown areas is characteristic of the Ayrshire breed.

A

B

C

A

B

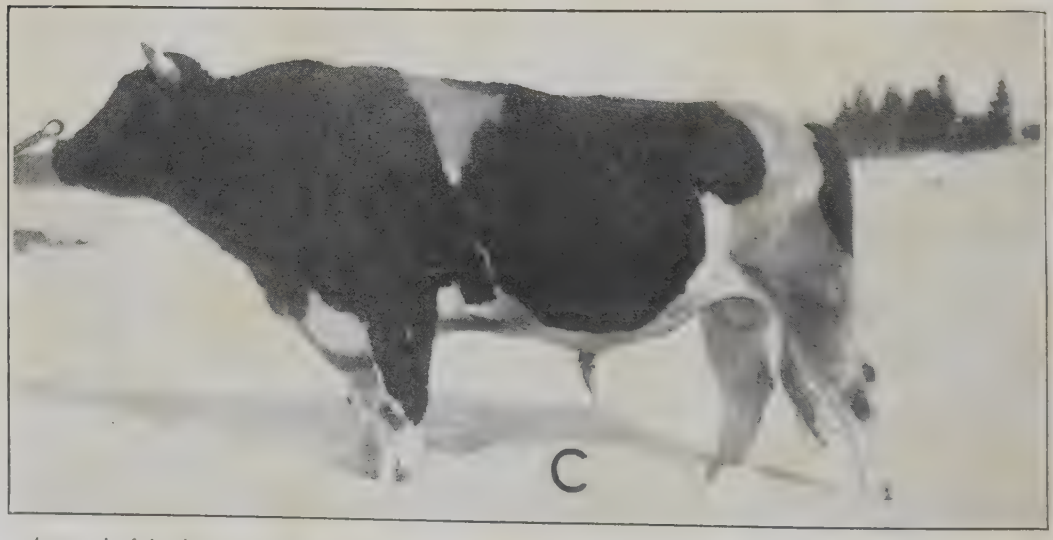
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PLATE 2

A.—Lady Primrose's Governor of the Fountain: This imported bull presents the characteristic conformation and white markings of the Guernsey breed. Notice the sag in the back and the high tail set.

B.—Creusa's Lady: The illustration of this Guernsey cow is inserted to show the large areas of white interspersed with the cream-colored hair typical of the coat of this breed. The presence of the star is quite characteristic.

C.—This Holstein-Friesian bull is of excellent constitution and vigor. He is the father of a good number of our crossbreds. Note that most of the white areas that are studied individually in this paper are present in this bull.

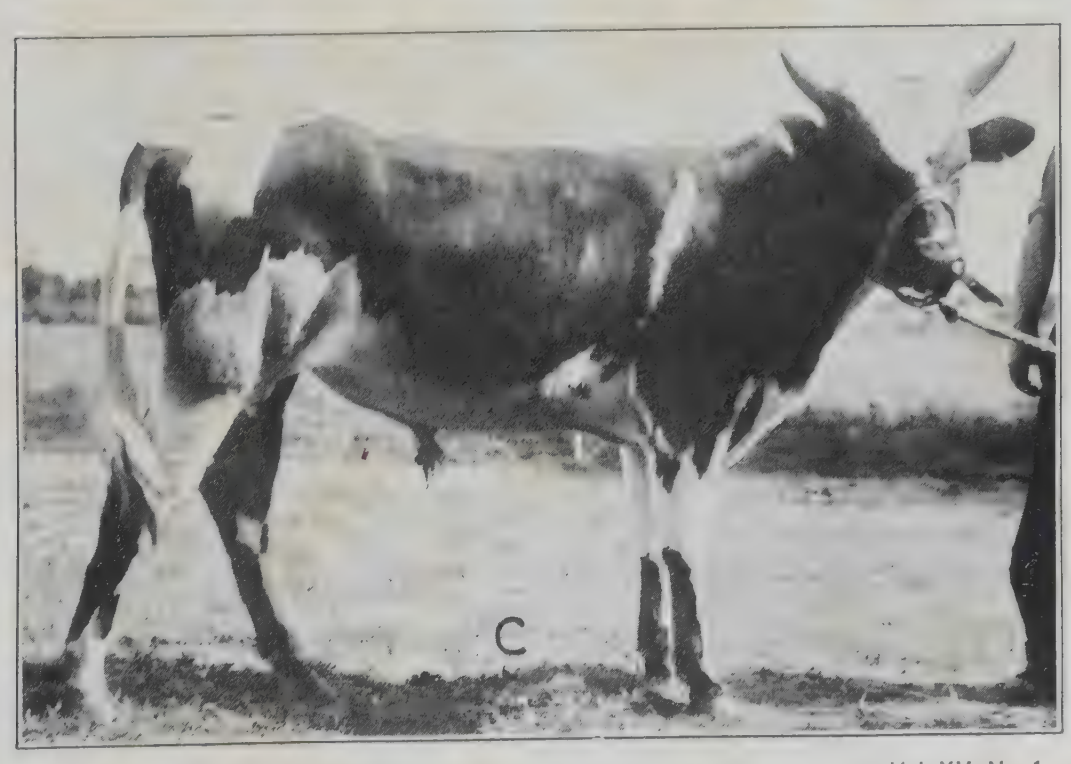
PLATE 3

A.—Lakeland's Poet: This Jersey bull exhibits the dark type of pigmentation at one end of the range of coat colors characteristic of the breed. He is at the opposite end of the range from Lassie of M. F., who shows the light type. He is the father of a number of the crossbreds now in the crossbred herd.

B.—Lassie of M. F.: The light pigmentation of the coat of this Jersey exhibits one end of the range of coat colors characteristic of the Jersey breed. The other extreme in pigmentation is shown by Lakeland's Poet (Pl. 3, A). The well-filled condition of the udder is typical of this Jersey.

C.—Crossbred 0: The characteristic shape of the head and carriage of horns show plainly the Ayrshire blood of this F_1 bull from a Holstein-Friesian × Ayrshire cross. The white markings and high cut hind legs do much to accentuate the Ayrshire appearance.

B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A

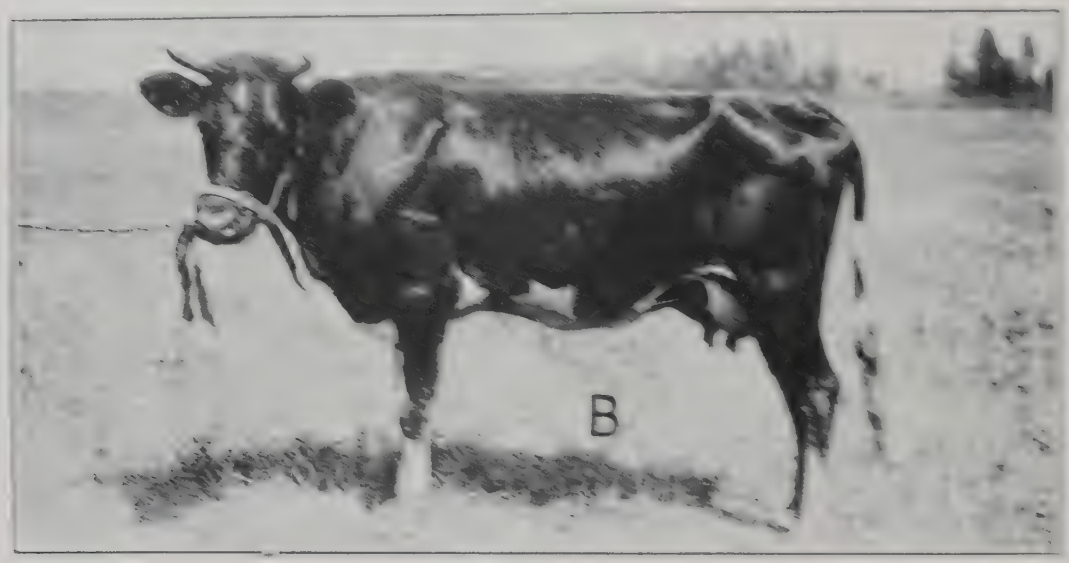
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PLATE 4

A.—Crossbred 9: This crossbred is essentially of the beef type. The Holstein-Friesian blood could scarcely be noticed except, perhaps, in the slightly high cut hind quarters. The characteristic scurs of the Angus crossbred male are easily noted.

B.—Crossbred 11: This animal is distinctly an intermediate between the Holstein-Friesian and the Jersey. The dish of the face and the thin nose and rump show plainly the Jersey. The parallel lines of the back and belly show the Holstein-Friesian influence.

C.—Crossbred 14: Another Holstein-Friesian-Jersey cross; this time a bull. The picture shows that the intermediate type is transmitted to the male as well as to the female.

PLATE 5

A.—Crossbred 15: This F_1 female out of a Jersey × Aberdeen-Angus cross shows the characteristic polled condition of the females of the Aberdeen-Angus crosses. The body type is that of the well-fleshed Jersey rather than the Aberdeen-Angus.

B.—Crossbred 19: A typical freemartin born twin with No. 18. The polled condition with loose bursas under the skin is interesting. The Angus blood is much more in evidence than the Jersey, as seen especially in the rounded condition of the body and heavy fore quarters.

C.—Crossbred 23: A Holstein-Friesian × Jersey bull, showing white hind feet and switch. He comes from a cross the male parent of which has the marks and the female of which is solid color. The pedigree of this female shows that she probably carries these marks.

A

B

C

A

B

PLATE 6

A.—Crossbred 38: This F_2 bull comes from the cross of a black F_1 bull Aberdeen-Angus-Guernsey × Guernsey. He is solid orange in color, carries horns, the light eye ring, and muzzle color of the Guernsey breed. The conformation resembles the Guernsey, especially in the region of the loin, chine, and tail set.

B.—Crossbred 21: This bull is the progeny of Kayan (Aberdeen-Angus clean-polled bull) mated with Dot Alaska (Ayrshire). Note the heavy, solidly attached horns grown while only a year and four months old. The Aberdeen-Angus blood is plainly seen in the heavy, beefy conformation of this bull. The other horned animal had horns even longer than these at this age.

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CONDITION OF FERTILIZER POTASH RESIDUES IN HAGERSTOWN SILTY LOAM SOIL[1]

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INTRODUCTION

It has long been known that when solutions of potassium salts are brought into contact with clayey or loamy soils, the potassium is quite rapidly removed from solution by the soil, with or without replacement in the solution by other basic elements in chemically equivalent amounts; also, that by immediate washing of the soils thus treated a large part of the potassium they have acquired from the potassium-salt solution can be recovered from the soil.

It has furthermore been shown by numerous investigations that, when the potassium salt is introduced into a clay or loam soil in a solid, such as the potash salt of a commercial fertilizer, the potassium is quite promptly "fixed," or united with the soil solids and made stationary at the point of introduction; that the downward movement of the potassium into the subsoil is relatively slight, and that the loss by drainage is small. On the other hand, in a sandy soil the drainage loss may be large (*2*).[2]

The testimony concerning the usefulness to crops of the potash thus fixed in the soil, the crop increases obtained in many instances by potash fertilization, and also the quantity of potassium taken up by the crop to which the fertilizer has been applied is that, whether a crop increase follows the potassium dressing or not, the crop is, in the majority of cases adequately studied, richer in potassium than the crop grown simultaneously upon the same soil without such fertilization. In general, the amount of potassium taken up by the crop fertilized with potash as contrasted with that not so fertilized is proportionally much greater than the increase in the total potassium supply of the soil due to the potassium dressing supplied.

[1] Approved for publication, by R. L. Watts, Dean and Director, Pennsylvania Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 81.

There is, indeed, much evidence that the crop fertilized never takes off potassium in amount equivalent to that contained in the usual fertilizer dressing; nor, very often, half as much. The agricultural value of the residual fertilizer potash is consequently a point of much economic importance, especially in view of present prices for fertilizer potash.

The final criterion of such value is, of course, in the crop yields and their potassium content noted at successive periods after potash fertilizing. There are of record few long-continued field or pot studies upon this point.

Chemical methods of soil examination are doubtless not strictly comparable with biological methods for determining the degree of "availability" of a plant food present in the soil, or for determining the quantity of such material present in different degrees of availability. They do serve, however, to determine the existence of differences in the condition of solubility of such plant food, and in a roughly quantitative way, the amounts present in the conditions contrasted.

PREVIOUS WORK

Numerous chemical studies of this sort have been made upon soils representing comparatively brief periods of fertilizer treatment with collectively small amounts of added fertilizer constituents. Few such studies represent, however, long periods of contrasted treatments definitely maintained. Of these, that reported by Dyer (*3*) from the examination of the barley soils of the Hoos Field, Rothamsted, is in many respects the most important. The treatment contrasted had continued for 38 years, and not only the amount and composition of the fertilizers but also the crop yields and the ash content and composition of the crops removed, were known for two of the plots, soils from which were analyzed. Of these two plots, No. 2A had received no fertilizer potash, No. 4A, 4,100 pounds. The potash had not largely increased the crop yields, but had increased the potash content of the barley; so that, while the 38-year yields from plot 2A contained 984 pounds of potash, those from plot 4A contained 2,057 pounds. The net residuum of fertilizer potash on plot 4A was, therefore, 2,043 pounds; whereas, plot 2A had lost 984 pounds in the crops, making a net contrasted difference between the two plots of 3,027 pounds. The results of the chemical examinations of the two soils at the end of the 38-year period with respect to potash are summarized as follows:

Constituent.	Potash (pounds per acre).			Ratio, 2A:4A.
	Plot 2A.	Plot 4A.	Difference.	
Total potash (modification of Smith method).	36,376	43,301	6,925	1: 1.19
Potash dissolved by strong hydrochloric acid (method not accurately described).....	6,269	8,242	1,973	1: 1.31
Potash dissolved by 1 per cent citric acid acting for two weeks at room temperature.	57	753	696	1:13.21

Moreover, in five groups of plots from the same field, each with two plots receiving no potash and two that were periodically dressed with potash fertilizers, the soils of the potash-dressed pair invariably contained more total potash than those of the nonpotash pair; with a single exception, more potash soluble in hydrochloric acid; and in all cases much more potash soluble in 1 per cent citric acid, the average ratio for the nonpotash to potash-treated soils in this last case averaging 1 to 9.

PENNSYLVANIA STUDIES

The soils used for the present study represent plots 1 and 4, Tier II, of the General Fertilizer Experiments of the Pennsylvania Experiment Station, which have been maintained continuously since 1881. All the plots of Tier II were cultivated and cropped alike, the land being kept under the common Pennsylvania 4-course rotation of corn (*Zea mays*), oats (*Avena sativa*), wheat (*Triticum aestivum*), and grass (mixed timothy (*Phleum pratense*) and medium red clover (*Trifolium pratense*). Plot 1 received no dressings of any kind during this experiment. Plot 4 was dressed biennially, for corn and wheat, with 200 pounds of muriate of potash, so that, beginning with 1881, it had received 18 such dressings, equivalent to 1,800 pounds of potash (K_2O) up to the time of the sampling in 1916, when the land was in oat stubble. The latest of these dressings had been applied 14 months prior to the sampling.

From 1868 to 1881 the plots were used for certain cultivation experiments, plot 1 being plowed with a common plow for corn and wheat, plot 4 with a subsoil plow for corn, and a Michigan plow for wheat. Both plots were, however, cropped alike and were also fertilized alike and not at all heavily. Prior to 1868 the land was under general farm cultivation and cropping, the two plots forming part of the same field and doubtless having had the same farm history.

The weight of the acre 7-inch surface layers of the two plots was determined [1] at 22 points uniformly distributed over each plot, with details of method and result that have been elsewhere reported.[2] The air-dry (not oven-dry) weights of the respective surface 7-inch layers were: Plot 1, 2,091,662 pounds to the acre; plot 4, 2,036,449 pounds to the acre. The probable error of these determinations was less than 10,000 pounds to the acre.

The 22 subsamples obtained in two independent series from each plot in the course of the acre-weight determinations were supplemented by 80 other subsamples, likewise divided into two independent series for each plot, obtained by means of a soil auger. Both sets of subsamples were prepared in the same manner, by air-drying and sifting all of each

[1] All quantitative determinations reported were, unless otherwise specifically credited, made by Mr. ERB under the supervision of the senior author.

[2] FREAR, WILLIAM, and ERB, E. S. EXCAVATION METHOD FOR DETERMINING THE APPARENT SPECIFIC GRAVITY OF SOILS. To be published in Proc. Assoc. Off. Agr. Chem. 1917.

subsample successively through a 4-mm. and a 1/20-inch (1.27 mm.) sieve. The fine soils obtained from the latter sifting were in each case composited so as to represent the four series of subsamples obtained from each plot, and the composites were then submitted to partial analysis. The results of this study of sampling methods have also been elsewhere reported in detail.[1]

The net results for the proportion and amount of fine soil (passing a 1/20-inch sieve) in the surface of the two plots, which contain a good many cherty fragments, were:

	Plot 1.	Plot 4.
Fine soil (per cent of air-dry sample).....	90.39	91.59
Fine soil (pounds to the acre 7 inches).....	1,890,644	1,865,947

The analyses of the several series composites from the same plot show composition differences somewhat greater than appear in duplicate analyses of the same composite. These series differences are rarely more than double the differences shown by duplicate determinations upon the same composite. They are not of such magnitude as to approach the differences exhibited by the analytical figures, either detailed or average, for the respective plots. For this reason the several sets of detailed analyses are not presented in full in the present account; but, when the repetitions of a determination upon composites from the same plot are sufficiently numerous, they have been used, by application of the Gauss formula, to calculate the probable error of the determination.

Because of the exceptional care employed in taking, preparing, and compositing the samples, it is believed that the materials used for this study are in an unusual degree representative of the soil areas from which they were obtained.

All analytical results stated in this paper are expressed in terms of the air-dry soil, without recalculation to a water-free basis. Determinations of hygroscopic moisture made when the analytical work was begun showed a range of 0.785 to 1.088 per cent for the eight series composites, with plot averages of 0.816 and 0.829 per cent, respectively. Another set of such determinations made about the close of the analytical work showed an average increase of only 0.05 per cent of moisture in the soils.

TOTAL POTASH

Portions of the series composites were reduced to an impalpable powder by grinding in an electric mortar mill of agate. The total potash content in each was determined in duplicate by the J. Lawrence Smith method as described by Washington (*o*, *p.* 129). The final weights of potassium platinichlorid were ascertained by first weighing the dried precipitate in a Gooch crucible, then washing out the soluble

[1] FREAR, WILLIAM, and ERB, E. S. A STUDY IN SOIL SAMPLING. To be published in Proc. Assoc. Off. Agr. Chem. 1917.

salt with hot water, and again weighing, after drying at 100° C., the crucible with its felt and any residual, insoluble impurities, of which a small amount always appeared. The difference between the two weights thus obtained was counted as the weight of potash salt. This procedure was used in all other potash determinations here reported.

The results of eight determinations of total potash for each plot were:

	Per cent.
Plot 1, untreated	3.821 ± 0.0240
Plot 4, potash dressed	3.543 ± .0134

The fact, curious at first blush, appears that the potash-treated plot contains at this time actually less potash than the untreated plot. The probable explanation of this condition is found in the rather high, natural variability in composition of the soils of the general series of plots, which has been established by other studies of these soils (*4, p. 187*).

POTASH SOLUBLE IN HOT, STRONG HYDROCHLORIC ACID

The method followed was substantially the old official method [1] as prescribed for cases in which only the alkalis are to be determined—that is, 10 gm. of the air-dry fine soil in its natural condition of subdivision were exposed for 10 hours on steam cups or in water bath to the solvent action of 100 cc. of hydrochloric acid (1.115 sp. gr.), with hourly shaking. The heavy metals and alkaline earths were thrown out of the solution by barium hydrate, and the excess of this precipitant was removed from the filtrate as oxalate. Each of the precipitates was washed with from 600 to 700 cc. of warm water to insure the complete recovery of the alkalis therefrom. All subsequent details of procedure designed for determining the sodium in the solution were omitted.

It is a matter of common experience that, of all the more abundant soil elements, potassium reacts most sensitively to the conditions of acid solution. Hilgard (*6, p. 342*) has emphasized this fact, and Frear and White (*4, p. 187*) have demonstrated it for the potash in the soils of the general fertilizer tract.

The temperature condition is especially influential upon the rate of solution for this constituent. In the first set of these determinations the dissolving flasks were heated on steam cups. While the solutions obtained gave in most cases fairly concordant duplicates, in others the differences between duplicates were equal to fully 20 per cent of the potash dissolved. These differences are attributed to inequalities of the temperatures maintained on the different cups, and in part also to the differences in the agitation of the liquids on different cups.

Anothet set of determinations was made later, in which the dissolving flasks, provided as in the former set with reflux condensers, were sus-

[1] Report of Committee on Editing Tentative and Official Methods of Analysis [Association of Official Agricultural Chemists], p. 24–25. 1916.

pended in a water bath to such depth that the level of water in the bath was somewhat higher than that of the acid in the flasks. The water was at boiling temperature (about 99° C.) when the flasks were introduced, and was so maintained during the solution process. The water level in the bath was maintained by frequent, small additions of water, and the flasks given a rotary shaking each hour. The bumping of the bath insured, however, a constant agitation of the soil patricles in the acid solvent. The maximum difference between duplicates observed when this solution method was followed was equivalent to no more than 3 per cent of the potash dissolved.

Plot and treatment.	On steam cups.	In water bath.
	Per cent.	*Per cent.*
Plot 1, untreated	[a] 0.2725±0.00346	[c] 0.3687
Plot 4, dressed with potash	[b] .3110± .005	[d] .4072±.00146

[a] 10 determinations.
[b] 16 determinations.
[c] Duplicate determinations.
[d] 6 determinations.

The water-bath treatment gave in each case about one-third more potash than was obtained by heating in the steam cups. Because the prescribed conditions were more certainly maintained by use of the water bath, and also because the results thus obtained are the less variable, these results are accepted as the better representing the method.

POTASH SOLUBLE IN WARM N/5 HYDROCHLORIC ACID

For this determination 100 gm. of the fine soil was used in its natural condition of subdivision, with 1,000 cc. of *N*/5 hydrochloric acid. The dissolving flasks were maintained at a temperature of 40° C. in an electric oven for five hours, and were well shaken at hourly intervals during that period. The solutions were then promptly separated from the undissolved soil by filtration. A 500-cc. aliquot of the filtrate was evaporated down for analysis. The details of analysis of the solutions were as previously described for the solutions otherwise obtained.

There were eight determinations for each plot, with results as follows.

	Percentage of potash (K_2O).
Plot 1, untreated	0.0143±0.00017
Plot 4, dressed with potash	.0301± .00019

POTASH SOLUBLE IN DISTILLED WATER

Determinations were attempted by three different methods of solution, with different ratios of soil to solvent, all at moderate temperatures, of the potash that could be dissolved by distilled water from the respective fine soils in their natural state of subdivision.

FLASK METHOD.—A 10-gm. portion of the soil was heated with 1,000 cc. of freshly boiled and cooled distilled water in an electric oven at 40° C. for five hours, with vigorous shaking at hourly intervals. At the end of the heating period the solution was filtered quickly away from the undissolved soil, and freed from suspended silt and clay by repeated filtration. The entire filtrate, after it had been freed from visible suspended matter, was concentrated by evaporation. To destroy the dissolved organic matter, the solution was evaporated to dryness, ignited, and the residue taken up with dilute acid. The determinations of potash were made gravimetrically as in the preceding solutions, but with highly variable results. Although the results from the respective soils, obtained by five repetitions for plot 1 and eight for plot 4, were—

	Percentage of potash (K_2O).
Plot 1, untreated	0.0032±0.0003
Plot 4, dressed with potash	.0049± .0006

the ranges of variation were, for plot 1, from 15 to 46 parts of potash per million of the soil, and for plot 4, from 17 to 75 p. p. m.

PERCOLATION METHOD.—For this method 50-gm. composites for each plot were gradually filled into 7/8-inch percolation tubes, closed below by means of a double paper filter reenforced by a linen filter firmly bound around the lower end of the tube. The soil was lightly tamped as it was filled into the tubes, so thot in each case the dry soil column had a final depth of 3¾ inches. The distilled water was delivered from a Bunsen bottle suspended over the tube at such rate as to maintain a 1-inch water head above the level of the light, acid-extracted asbestos wad placed over the soil to secure uniform entry of the liquid into the soil column and to prevent spattering and superficial packing of the soil. Four such tubes containing duplicate portions of the two soils were supported side by side at room temperature (about 21° C.), under like evaporation conditions, and the percolation was continued until 500 cc. of percolate had been obtained from each. The times required for the percolation differed somewhat.

The percolates were filtered until free from clay, and potash was then determined as in the preceding solutions, with the following results:

Plot.	Treatment.	Set.	Percolation tim .	Potash.	
				Found.	Average.
			Days.	*Per cent.*	*Per cent.*
1	Untreated	a	4	0.0048	0.00455
1	do	b	4	.0043	
4	Dressed with potash	a	4	.0083	.00800
4	do	b	6	.0077	

CLAY SEPARATION WATER.—For a purpose stated later, a mechanical separation of the clay from these soils was made in the following manner: Six hundred gm. of the air-dry fine soil was shaken with about 2 liters of distilled water in a rotary shaker for eight hours. After the suspensions thus obtained had settled overnight, the clayey liquor was carefully siphoned off. The soil residues were then thoroughly agitated with a fresh portion (1.5 to 2 liters) of distilled water, then allowed to stand until the particles of more than 0.005 mm. in diameter had settled out, when the new clay suspension was siphoned off. This washing process was repeated for 10 days. Even then not all the clay had been removed from the coarser soil members. The volumes of the combined washings were, for plot 1 soil, 16,710 cc.; for plot 4, 18,530 cc.

To remove the clays from these suspensions they were allowed to stand for two days, by the end of which five-sixths of the clay had settled out. The overlying liquors were carefully drawn off, and the clay still remaining in suspension in the liquor was almost completely removed by flocculation. The flocculating material first used was ammonium chlorid. This was added in 5-gm. portions previously dissolved in a little distilled water, and thoroughly stirred through the liquor.

In the case of the suspension from plot 1 soil, flocculation began shortly after the addition of two portions, or 10 gm., of the flocculant; but three portions, or 15 gm., caused no appearance of flocculation in the suspension from plot-4 soil. This liquid had the appearance which slight alkalinity gives to clay suspensions, although rather sensitive pink litmus gave no alkaline reaction when moistened with it. The addition was tried, therefore, of concentrated hydrochloric acid, drop by drop, with stirring after each drop had been added. Upon the introduction of the tenth drop, flocculation began rather sharply, so that the addition of the acid was discontinued.

The liquors drained off from these flocculated clays were still faintly cloudy, and separated a little clay on long standing.

Of the washings from plot 1, 14,530 cc., and of those from plot 4, 13,030 cc. were withdrawn, filtered, evaporated to dryness, and the evaporation residue ignited to get rid of organic matters and ammonium salt. The ignited residues were moistened with acid, taken up with hot water, filtered, and subjected to analysis for potash by the method already described.

The quantities of potash found in the total wash waters were:

	Percentage of soil.
Plot 1, untreated	0.0038
Plot 4, dressed with potash	.0087

In view of the differences in method of treatment, in proportion of soil to water, and in duration of the exposure of soil to solvent, it is remarkable that the quantities of potash removed by water were in all cases so nearly the same for the respective soil samples.

POTASH SOLUBLE IN CARBONATED WATER

The quantities of soils used and arrangement of apparatus were substantially the same as those in the percolation with distilled water. The principal modifications consisted in (1) the delivery of water charged with carbon dioxid at air pressure and room temperature by means of a continuous current of the gas passed through the water in the Bunsen bottle; and (2) the closing of the top of the percolator tube by means of a one-hole rubber stopper, to prevent loss of the gas from the apparatus at that point.

The percolations were continued until in each case 500 cc. of percolate had been extracted. The linen filter was washed in both percolation tests with a spray of water to remove any soluble film that might have been deposited by evaporation of the percolate. The washings were added to the latter.

The usual analytical procedure was followed, with these results, in terms of the air-dry soil:

Plot.	Treatment.	Set.	Time of percolation.	Potash (K_2O).	
				Found.	Average.
			Days.	*Per cent.*	*Per cent.*
1	Untreated	a	5	0.0088	0.0076
1	do	b	2	.0064	
4	Dressed with potash	a	4	.0162	.0140
4	do	b	3	.0123	

While the percolates which passed more slowly through the soil contained more potash than those which passed quickly, the increase was not proportional to the time of percolation for either soil.

POTASH SOLUBLE IN AMMONIUM-CHLORID SOLUTION

One hundred gm. of the air-dry fine soil in its natural state of subdivision were heated with 1,000 cc. of the neutral-salt solution for five hours in an electric oven at 40° C. with hourly shaking. At the end of the heating period the liquors were immediately filtered off from the undissolved soils, and subjected to the analytical procedure previously described.

To find a satisfactorily active solution concentration of the ammonium chlorid without too greatly increasing the amount of this salt that must later be removed in the analytical process, the effects of solutions containing 17.6 and 50 gm., respectively, of the salt in 1 liter of distilled water (approximately *N/3* and normal solutions) were compared

with that of distilled water. The results, in terms of air-dry soil, obtained with a sample from plot 4, were:

Solvent.	Set.	Potash.		
		Found.	Average.	Excess over water effect.
		Per cent.	*Per cent.*	*Per cent.*
Distilled water	a	0.0075	0.0073	
	b	.0070		
N/3 ammonium-chlorid solution	a	.0194	.0190	0.0177
	b	.0186		
Normal ammonium-chlorid solution	a	.0220	.0216	.0143
	b	.0212		

This shows that, under the conditions maintained, the *N/3* solution had about 2.7 times the solvent effect of water alone; and the normal solution about 3.1 times that of water. In other words, trebling the concentration of the salt solution increased its solvent effect little more than one-eighth.

Hence, as the *N/3* solution introduced no more ammonium chlorid than could be handled with a fair degree of convenience in the analytical operations, a solution of that concentration (17.6 gm. of salt to 1,000 cc. of water) was used in the remaining studies with the solvent.

The average results for nine samples of each soil were, in terms of the air-dry soil and corrected for blank:

	Percentage of potash (K_2O).
Plot 1, untreated	0.00936±0.00012
Plot 4, dressed with potash	.01872± .00039

In order to ascertain the relative solvent effect of a second extraction with this solution after two of the treatments, one for each plot, included in the above averages, the once-extracted soil residues were carefully washed with a little water to remove the excess of the salt solution, dried on the filter, and again subjected, as before, to the action of 1 liter of the fresh salt solution. The results obtained in the respective extractions were:

	Percentage of potash (K_2O).
Plot 1, first extraction	0.01002
Plot 1, second extraction	.00198
Plot 4, first extraction	.02210
Plot 4, second extraction	.00208

The interesting fact appears that the amounts of potash removed in the second extraction were almost identical for both soils, and in both cases were much less than the quantities removed by the first extraction. In order to check this finding, a second portion of plot-4 soil was

thus extracted a second time, with a recovery (net) of 0.00190 per cent of potash in the second extract.

It happens that the quantity of potash removed by the two extractions of the soil from plot 4 was practically the same as the amount removed by a single extraction with ammonium-chlorid solution of normal concentration.

Summary of fine-soil potash solubilities

Treatment of potash.	In terms of air-dry fine soil.		In terms of total potash.	
	Plot 1.	Plot 4.	Plot 1.	Plot 4.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Total	3.821	3.543	100.000	100.000
Soluble in hot (1.115 sp. gr.) hydrochloric acid, 10 hours, 1:10	.3687	.4072	9.649	11.493
Soluble in warm (40° C.) *N*/5 hydrochloric acid, 5 hours, 1:10	.0143	.0301	.374	.850
Soluble in distilled water:				
Flask method, 5 hours 40° C., 1:10	.0032	.0049	.084	.138
Flask method, highest results	.0046	.0075	.120	.212
Percolation method, 21° C., 1:10, 4 to 6 days	.0045	.0080	.118	.226
In water from clay washing	.0038	.0087	.099	.246
Soluble in carbonated water by percolation, 21° C., 1:10, 2 to 5 days	.0076	.0140	.199	.395
Soluble in *N*/3 ammonium-chlorid solution, 5 hours, 40° C., 1:10:				
First extraction	.0094	.0187	.246	.528
Second extraction	.0020	.0021	.052	.059

In general, only about one-tenth of the potash of these soils can be extracted from the fine soil in its natural state of subdivision by strong, hot hydrochloric acid acting at the temperature of boiling water for a period of 10 hours, when 10 cc. of the acid are used for each gram of the soil.

When the conditions of solution are closely maintained, the potash of the potash-fertilized plot is almost one-fifth more soluble in strong, hot acid than that from the untreated plot.

When mild solvents are used to extract the potash, the differences in the condition of this element in the two soils are much more pronounced. The solubility of the potash in the potash-fertilized plot is from 1.5 to 2.5 times greater than in the untreated plot.

The similarity of the ratios between the percentages of potash dissolved from the two soils by the respective mild solvents, distinctly suggests identity in the nature of the materials acted upon in the soils by these solvents. On assuming that water and its solutions of carbon dioxid and of ammonium chlorid act only upon those potassium compounds of the soil that weak hydrochlorid acid can attack, and expressing the

amounts of potash dissolved by the former as percentages of the entire amount dissolved by the *N/5* acid, and in this comparison taking as typical of the water action the results obtained by percolation, because they are internally the most consistent, the percentages thus computed are:

Treatment of potash.	In terms of potash soluble in *N/5* hydrochloric acid.	
	Plot 1.	Plot 4.
Dissolved by—	*Per cent.*	*Per cent.*
Distilled water (by percolation)	31.6	26.6
Carbonated water (by percolation)	53.2	46.5
Ammonium-chlorid solution, *N/3*	65.5	62.1

There appears a very close similarity between these percentages for the two soils. The figures for plot 4 lag somewhat behind those for plot 1, but probably no more than should be expected when the slow solution rate here controlling and the much larger amount of soluble potash in plot 4 are considered. In other words, there is here a close parallelism between the solvent effects with regard to potash of the *N/5* hydrochloric acid and of the other weak solvents.

We may infer from these facts of chemical behavior that the residues from potash fertilization remain, at least in considerable measure, in a state of availability in the surface soil to which the fertilizer was applied. The final arbiter of availability is the plant itself. Certain plant evidence in our possession with respect to the availability of the potash in the soils under the treatments here contrasted will be presented later in this paper.

POTASH OF THE CLAYS AND NONCLAYS OF THE TWO SOILS

With the purpose of examining the condition of the potash in the two soils from another viewpoint, the clays were separated from the nonclays by the method of sedimentation in distilled water. The details of the process are given in the paragraphs dealing with the water solubility of the potash.

The clays and nonclays from each plot were assembled, with slight mechanical losses, on containers and filters, air-dried, and weighed.

The net results of the separations were:

Group.	Plot 1, untreated.		Plot 4, potash-dressed.	
	Weight.	Per cent.	Weight.	Per cent.
	Gm.		*Gm.*	
Clay, air-dry	70	11.67	66	11.00
Nonclay, air-dry	511	85.17	517	86.17
Solution and mechanical losses	19	3.16	17	2.83
Total	600	100.00	600	100.00

For a check upon the accuracy of the foregoing results obtained by the separatory treatment of relatively large quantities of soil, mechanical analyses of grand composites of the series composites for the respective plots were made by Mr. Walter Thomas, of this laboratory, using the Bureau of Soils methods and types of apparatus, upon 6-gm. portions of the fine soils. His results were:

Group.	Diameters.	Plot 1.	Plot 4.
	Millimeters.	*Per cent.*	*Per cent.*
Fine gravel	2 −1	.16	.33
Coarse sand	1 − .5	2.28	2.81
Medium sand	.5 − .25	2.86	2.90
Fine sand	.25 − .1	.56	.70
Very fine sand	.1 − .05	14.90	14.05
Silt	.05 − .005	63.50	63.46
Clay	.005− .0	10.08	10.24
Ignition loss	.....	5.65	6.06
		99.99	100.55

These percentages differ somewhat from those reported earlier by Brown and Skinner (*1*, *p. 30*) for the surface soils of these plots, who found in brief:

Group.	Plot 1.	Plot 4.
	Per cent.	*Per cent.*
Fine gravels and sands	11.9	15.9
Silt	73.6	69.5
Clay	13.9	13.9
Total	99.4	99.3

That is, they found less sand and more silt and clay, although their disregard of ignition losses somewhat increased their percentages.

In general, the figures obtained by the sedimentation of 600-gm. portions of the soil are essentially confirmed by the mechanical analyses.

The two soils are practically alike in their proportions of the mechanical components. The observed difference in the apparent specific gravity of the soils is probably the consequence chiefly of some unlikeness in the arrangement of the constituent soil grains.

THE CLAYS

The characters of the separated "clays" were distinctly claylike. When moist, they had a putty-like consistence; and, when dried, were hard and broke with a gluelike fracture. Microscopic examinations of the suspensions before the final sedimentation showed that most of the particles, visible under a one-twelfth objective, had diameters less than 0.0025 mm.

Portions of the clays were analyzed by the J. Lawrence Smith method, with these results, in terms of the amounts of clay:

	Percentage of total potash in clays.
Plot 1, untreated	3.310
Plot 4, dressed with potash	3.137

The clay from plot 4, as with the entire fine soil, contained less potash than the corresponding fraction of the plot 1 fine soil. In each case, however, the clay contained a less percentage of potash than appeared in the total fine soil.

THE NONCLAYS

The total potash in the washed nonclays was not determined directly. Computed by difference, the percentages are, in terms of the amounts of nonclays:

	Percentage of potash in nonclays.
Plot 1, untreated	4.027
Plot 4, dressed with potash	3.701

SOLUBILITIES OF THE POTASH OF THE WASHED CLAYS AND NONCLAYS IN *N*/3 AMMONIUM-CHLORID SOLUTION

The washed clays and nonclays thus separated and air-dried were treated in 50-gm. portions with 500 cc. of *N*/3 ammonium-chlorid solution for five hours at 40° C., with hourly shaking, and the resultant solutions were immediately filtered off and analyzed for potash. The results are stated below in terms of the total weight of clays and nonclays, respectively:

Potash dissolved from—	Plot 1, per cent.	Plot 4, per cent.
Clay, washed	0.0352	0.0408
Nonclay, washed	.0041	.0050

We may safely conclude from these studies that, although the clays are not so rich as the nonclays in total potash, weight for weight, they yield to weak solvents from six to eight times as much of this element during a short period of time. It is also clear that, while both fractions of the potash-dressed soil are richer in soluble potash than the corresponding fractions from the unfertilized soil, the clays differ in this particular much more than the nonclays.

The percentages of the total potash in these washed clays and nonclays that are removed by the ammonium-chlorid solution are:

Potash dissolved from—	Plot 1, per cent.	Plot 4, per cent.
Clay, washed	0.761	1.306
Nonclay, washed	.102	.132

That is, whether the amount dissolved be expressed in terms of the respective soil fractions or of their total potash contents, the solubility of the clay potash is much greater than that of the nonclay.

RELATION OF AMOUNTS OF POTASH DISSOLVED FROM CLAY AND NONCLAY TO THE COLLECTIVE SURFACE AREAS OF THEIR PARTICLES

Since one of the important factors determining the quantity of a given solid that can be dissolved by a given solvent is the amount of surface which the solid exposes to the liquid, it is worth while to attempt an approximation of the surface areas exposed to the solvent by the clays and nonclays of these soils, and to the amount of potash dissolved from unit areas respectively exposed to the solvent.

In the following tabular statement are given the areas, in square meters, of the clay and nonclay fractions in 100 gm. of the respective air-dry soils, as calculated by the conventional method (7, *p. 118*), using the formula

$$\text{Surface area} = \frac{6 \times w}{d \times 2.65}$$

in which "w" is the weight in grams of the group of soil particles for which the surface area is to be computed; "d," the mean diameter, in centimeters, of the particles of the group; and "2.65" the assumed specific gravity. The areas stated for the nonclays are the sums of those separately computed for the particles of each class as to fineness, other than the clays. Owing to the practical identity in mechanical composition of the soils of the two plots, the same percentages of mechanical composition were used in the calculations for both soils. With these areas (expressed in square meters in the table) are given the corresponding quantities in milligrams of the potash dissolved by the ammonium-chlorid solution from 1 square meter of the particle surface of the respective fractions.

If we accept without qualification these results of the conventional computation of the surface areas of the respective groups of soil particles,

we are faced with the apparent fact that the potash of the clay is much less soluble, in proportion to the quantity directly exposed to the solvent, than that of the nonclay. This would tend to negative, at least for these two soils, the widely held theory that the soil particles are covered with a fairly homogeneous celloid coating from which weak solvents take up most of the materials they gain from the soil in a short time after they begin to act upon it; or else, it must be assumed that such coatings are much thicker on the nonclay than on the clay particles.

Item.	Clay.	Nonclay.
Surface areas of fractions in 100 gm. of soil.....square meters..	9.1	5.7
Potash (milligrams) dissolved by *N*/3 ammonium-chlorid solution from 1 square meter of particle surface:		
Plot 1, unfertilized.....	.28	.61
Plot 4, dressed with potash.....	.45	.74

It is here needful, however, to qualify our acceptance of these conventional estimates of the surface areas of the particles acted upon.

It was noted in an earlier paragraph, that the larger proportion of the clay particles, when their watery suspensions were examined microscopically, showed diameters less than 0.0025 mm., the mean diameter for the clay group. The effect of the average diameter being in these cases less than the mean diameter for the clay group is, of course, to increase, possibly in quite large degree, the corresponding surface areas and to diminish correspondingly the quantity of potash dissolved from a unit area.

On the other hand, the portions of the washed clays and nonclays submitted to solvent action had been air-dried after separation by sedimentation. A portion of each of the air-dried clays was shaken up with water to see whether the drying had resulted in any flocculation or cementing of the particles. It was found difficult to get all the clay back into suspension, even with the aid of a little ammonia added to deflocculate. Still, the proportion of the dry clay not readily taken back into suspension was relatively small. Another condition, possibly much more highly determining in its effect, is found in the fact that ammonium-chlorid solution itself acts as a flocculant, so that a free exposure of the surfaces of the clay particles is impossible in its presence.

POTASH IN CROPS HARVESTED FROM THE UNTREATED AND POTASH-DRESSED LANDS

No studies of the potash content of the crops from plots 1 and 4 of Tier II were made in connection with the present investigations. There are available, however, two sets of analyses made of crops harvested at different times from the plots of corresponding numbers and history

belonging to Tiers III and IV, of the same General Fertilizer Experiments. The former of these sets of analyses was made by Pingree (*8*), using the oat crop harvested in 1904 from Tier IV. The second set is composed of unpublished analyses made by senior students of the Department of Agricultural Chemistry of The Pennsylvania State College under the direction of Dr. C. W. Stoddart, who has kindly permitted the use of the results obtained. This second set of analyses represents the crops of a complete rotation, 1910–1913, from plots 1, 14, 26, and 36 (untreated) collectively, and from plot 4, dressed with potash, all of Tier III.

The following is a condensed summary of these crop analyses, with respect to the potash found:

Crop.	Untreated.		Dressed with potash.		Ratios of potash percentage in crop of plot 4 (plot 1=100).
	Weight of crop.	Potash.	Weight of crop.	Potash.	
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	
Oats, 1904 (Tier IV)	2,320	26.17	2,360	38.86	146.3
Corn, 1910 (Tier III)	2,525	15.37	2,788	19.74	115.3
Oats, 1911 (Tier III)	832	9.81	1,040	18.01	147.0
Wheat, 1912 (Tier III)	2,669	12.16	2,172	11.97	120.6
Hay, 1913 (Tier III)	1,140	10.30	1,040	16.50	175.6
Total for 5 crops	9,486	73.81	9,400	105.08	143.7
Total for rotation, 1910–1913	7,166	47.64	7,040	66.22	141.9

In every one of these crops the percentage of potash was very markedly greater in that grown upon the plot dressed with potash, than in the corresponding crop grown upon the untreated plot 1, or upon its similars in treatment, plots 14, 26, and 34. The excesses on plot 4 for each unit weight of crop harvested varied from 15.7 to 75.6 per cent, and averaged 43.7 per cent for the five crops analyzed; 41.9 per cent for the four crops of 1910–1913, representing one complete rotation.

Since the potash fertilizer was applied just before the planting of the corn and wheat crops, it is perhaps not unexpected that these crops as harvested contain more potash than crops from lands receiving no fertilizer dressing. It is strikingly clear, however, that the larger excesses appear in the oats and hay crops, harvested 14 and 21 months, respectively, after the potash dressing had been applied. Furthermore, the excesses of potash in the plot 4 crops are possibly the more indicative of the ready availability of the potash in the soil of that plot from the fact that the crop yields were not increased by the potash fertilization.

The evidence from plant composition confirms, therefore, the indications obtained by the action of weak solvents upon the soil to the effect that of the potash in plot 4 much more exists in a state of availability to plants than of the potash in plot 1 despite the greater absolute quantity of the element present in the latter plot.

FATE OF THE FERTILIZER POTASH APPLIED

For an exact statistical allotment of the 1,800 pounds of potash applied to plot 4 from 1881 to 1916, inclusive, there would be required a number of data which the previously related studies have not furnished. We know that there is little loss or gain of the surface soil of this plot by erosive influences. The drainage is reasonably free, but there is no water table, for the drainage waters creep along the faces and through the crevices and seams of the limestone rocks that lie only a short distance beneath the surface. It has not been practicable, therefore, to collect and examine the drainage water lost from these two plots so as to determine the amounts of potash they severally lose through that channel. The general composition of drainage waters does not suggest that this loss can be large.

We have, moreover, no entirely satisfactory notion of the potash transfers from surface to subsoil on these plots. Little downward movement is indicated by the data at hand.

A crude approximation of the fate of the fertilizer potash is possible, nevertheless, from the data in hand if the losses by subsoil and drainage are treated as relatively small, and therefore negligible for the purpose of the computation.

The total yields (in pounds) to the acre, 1881–1916, for the respective rotation crops were, according to data supplied by Prof. C. F. Noll, of the Department of Agronomy of this Station:

Crop.	Plot 1, untreated.			Plot 4, potash-dressed.		
	Grain.	Straw.	Total.	Grain.	Straw.	Total.
Corn	18,144	14,784	32,928	21,072	19,000	40,072
Oats	8,002	11,799	19,811	9,236	13,215	22,451
Wheat	6,567	9,584	16,151	6,737	11,477	18,214
Hay	.....	.....	18,448	.....	.....	20,887
Total	.....	.....	87,338	.....	.....	101,624

If we estimate the potash removed by unit weights harvested of these several kinds of crops to have been, on the average, equal to the corresponding removals during the rotation 1910–1913, the total quantities thus taken from the two plots were:

Crop.	Percentages of crop.		Total weight (pounds).	
	Plot 1.	Plot 4.	Plot 1.	Plot 4.
Corn	0.609	0.708	200.5	283.7
Oats	1.179	1.732	233.4	388.9
Wheat	.456	.550	73.6	100.2
Hay	.904	1.587	166.8	331.3
	.....	.....	674.3	1,104.1
Excess removed from plot 4	.....	.....	.....	429.8

The excess removal by crops from plot 4 thus calculated is probably too great. The crop yields of plot 1 have been decreasing, but those also of plot 4 have been falling off at a similar rate. The potash stock of plot 1 has been drawn upon without replacement; that of plot 4 has been increasing. For, even at the rates apparent in 1910–1913, the average annual removals of potash in crops from the respective plots were: From plot 1, at the rate of 18.7 pounds to the acre; from plot 4, 30.7 pounds; whereas the average annual potash addition to plot 4 was 50 pounds to the acre. It is very possible, therefore, that the excess percentage of potash in a unit weight of harvested crop removed from plot 4 over that from plot 1 is now greater than in the earlier years of the experiment.

At the utmost, therefore, only 430 pounds of the 1,800 pounds of fertilizer potash applied to plot 4 has been removed in the crops harvested if, for this computation, we assume that the withdrawal from the original soil stocks were the same on each plot for each unit weight of a given crop harvested and that the excess removal on plot 4 is to be charged to the fertilizer potash. This leaves a balance of 1,370 pounds of fertilizer potash to be accounted for.

Upon the same basis of computation the crops of plot 4 used of the fertilizer applied only 12 pounds out of 50, or less than one-fourth. The smallness of this utilization is to be ascribed to the large natural supply of potash in this soil and the little crop increase the potash fertilizer here induces.

The actual weights of potash existing in the soils in different states of solubility remain to be considered. From data presented in an earlier section of this paper, the following facts appear as to the respective fine soil weights for the surface acre 7-inch layers and the total potash percentages:

	Plot 1.	Plot 4.
Weights of fine soil of acre 7 inches..pounds..	1,890,644	1,865,947
Total potash.....per cent..	3.821	3.543
Total potash.....pounds..	72,241	66,110

That is, the plot dressed with potash now contains, despite the fact that it received 50 pounds of potash a year, about 6,000 pounds less potash than the untreated plot. These differences in potash content of the two plots are doubtless due to their geological rather than their agricultural history. Their initial differences in potash content unsuit these two soils for any direct statistical comparison.

We may, however, assume that if plots 1 and 4 had had the same potash content at the outstart of the experiment and the same treatment since then the percentage proportion of this potash soluble under the conditions here studied would have been alike. To estimate the present differences in potash condition so as to exclude the influences of geological history and to represent only the effects of the differences in agricultural treatment, the respective solubility percentages for the potash in the contrasted plots are used as the quality factors and the present total potash in the plot 4 surface soil as the quantity factor. The present potash supply in the latter plot is slightly greater than it would have been if the potash additions had not been made. The residue of these additions is certainly not greater than 1,371 pounds, or approximately 2 per cent of the present potash stock. The influence of this amount upon these admittedly crude approximations has been deemed too slight for consideration in this computation. The potash weights thus computed to the acre are:

Treatment of potash.	Untreated soil.	Potash-dressed soil.	Excess in potash-dressed soil.
	Pounds.	*Pounds.*	*Pounds.*
Total potash	66, 110	66, 110	.....
Insoluble in hot, strong hydrochloric acid (1.115 sp. gr.) in 10 hours	59, 731	58, 512	.....
Soluble in 10 hours	6, 379	7, 598	1, 219
Soluble in *N*/5 hydrochloric acid, 5 hours at 40° C	247	562	315
Soluble in distilled water	78	149	71
Soluble in carbonated water	132	261	129
Soluble in *N*/3 ammonium-chlorid solution	163	349	186

It has already been observed that the natural stock of potash in the untreated soil is high. At the annual rate of removal in harvested crops for 1910–1913, the total supply would last 3,500 years, and that soluble in strong hot acid about 350 years. Even the quantities soluble upon a few hours' exposure of the soil to weak solvents would suffice for a number of years: The water soluble, 4 years; carbonated water soluble, 7 years; weak ammonium chlorid, 9 years; weak, warm acid, 13 years. Vigorous crops of normal quantity would, of course, remove more potash, but even for such growths the amounts readily soluble in the more or less carbonated soil moisture should amply suffice, judging from the observed solubility proportions. Experience on these lands has amply

demonstrated that only after years of normal crops have been removed is there any evidence of crop benefit by reason of potash applications.

The amounts of soluble potash in the soil that was dressed with potassic fertilizer are, no matter how weak the solvent used, relatively much greater than are found in the untreated soil. This fact, together with the composition of the crops grown on the respective lands, warrants the conclusion that the potash dressings remain, at least in part, readily available for some years after the application. The excess soluble in water corresponds to 1.4 times the annual addition; in carbonated water, 2.6 times; in weak solution of a neutral salt, 5.2 times; and in weak, warm acid, 6.3 times.

It is a curious coincidence that the excess of potash dissolved by hot, strong acid from the soil dressed with potash lacks very little of being equal to the amount of this element added, 1881–1916, less the excess quantity removed from plot 4 in the crops harvested (1,800–430 = 1,370 pounds). That this closeness of agreement is merely a coincidence must be evident from what has already been remarked concerning the large effects upon the strong acid extraction of soil potash that are observed when the time or temperature conditions are slightly changed. Frear and White (*4, p. 187*) have reported, from analyses of the sod lands adjacent to and intersecting the tiers of plots under the general series of experiments, that while the Association method of acid treatment removes somewhat over 8 per cent of the potash of these sod-land surface layers, Hilgard's method, which requires a 5-day instead of a 10-hour treatment, increases to 22.7 per cent the proportions of the total potash removed.

Finally, it should be noted that, although the crops harvested from plot 4 removed only two-ninths of the fertilizer potash applied, only three-eighteenths of the quantity applied remains in such condition that it can be readily dissolved by warm, weak acid. These quantities leave eleven-eighteenths of the applied amounts to be otherwise accounted for. The figures for potash soluble in strong acid do not indicate that much of this potash, except what the harvested crops took away, has been removed from the surface soil. Most of it remains there in rather difficultly soluble condition. In other words, the residual potash tends to assume, in large part, a condition of relatively slow availability. Concerning the time relations of this change, little is known. Gilbert (*5*) remarked a similar change in the solubility of the residues of fertilizer potash in the loam soil at Rothamsted.

SUMMARY

A comparison, as to the condition of the potash in a Hagerstown silty loam soil which has in the past 36 years received, in 18 equal biennial dressings, 1,800 pounds of fertilizer potash, with that in a neighboring

portion of the same soil which has, during the same period, received no fertilizer addition of any kind, but which has been tilled and cropped the same, gives the following results:

(1) The proportion of the potash dissolved by strong, hot acid is somewhat greater where the potash dressings have been used. In weak solvents (distilled water, carbonated water, weak solution of ammonium chlorid, and *N*/5 hydrochloric acid) twice as much potash is dissolved in a short time at moderate temperatures from the fertilized soil as these solvents take, under the same solution conditions, from the unfertilized soil.

(2) Of the weak solvents named, the *N*/5 acid dissolves the most potash. The quantities of this element dissolved by the other weak solvents, differ, of course, with the solvent; but each forms in each soil the same percentage proportion of the amount dissolved by the *N*/5 acid—that is, the solvent effects are parallel.

(3) The potash dissolved upon a second extraction with ammonium-chlorid solution is very much less than is dissolved by the first extraction with this solvent, and the quantities are practically the same for each soil; whereas that removed by the first extraction from the soil dressed with potash is twice as great as the amount taken from the unfertilized soil.

(4) The clays and nonclays of these soils, after separation by sedimentation in water, show the following characteristics as to potash content and solubility: The clays contain less potash than the nonclays. The clay of the potash soil is richer in potash than that of the unfertilized soil. The same is true, but in less degree, of its nonclay fraction. Unit weights of the clays give up much more potash to ammonium-chlorid solution than unit weights of the nonclays. Both fractions of the potash-dressed soil exceed those of the unfertilized soil in this respect, but the clays much more than the nonclays. Unit surface areas of the clay particles, as conventionally calculated, give up much less potash to the solution than equal surface areas of the nonclays. This may, however, be due to a reduction of free-clay surface to less than the conventional area by cementing of the particles in drying, or because of the flocculating influence of the saline solvent used.

(5) The soil is naturally rich in potash, and potash dressings cause little or no crop increase. These dressings are followed, however, by an increase in the amounts of potash taken up by the crops. Five crops examined all show this increase. On the average for a rotation, the crops harvested from the land dressed with potash carry off in a given weight of harvest, 40 per cent more potash than a like harvest weight from the unfertilized land contains—that is, both chemical solvent and plant agree in indicating a higher availability for at least part of the potash in the potash-dressed soil. Moreover, the crops grown the

second year after the application show a greater potash excess than those to which the potash fertilizer is directly applied.

(6) On crediting the fertilizer potash with the excess only of the potash in the crops from the fertilized soil, the crops have used not more than one-fourth of the potash dressings applied, leaving a residue of 1,300 to 1,400 pounds of fertilizer potash to be otherwise accounted for. The higher solubility in weak solvents of the potash in the fertilized soil accounts for enough of this residue to correspond to crop requirements through a few years. The larger amount of potash dissolved from the fertilized soil by strong, hot acid accounts for practically the entire residue, but the close correspondence in potash quantities here observed is doubtless an accident due to the particular conditions of temperature and duration of solvent action maintained.

(7) Taken as a whole, the conclusion is warranted that much of the potash applied as fertilizer remains in the surface soil in a state highly available to crops; that most of it remains there in a condition of lower availability, and that the losses by drainage have probably not been great.

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HARDENING PROCESS IN PLANTS AND DEVELOPMENTS FROM FROST INJURY [1]

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INTRODUCTION

Hardening plants to resist frost injury is a well-established practice. The physiological basis for this practice and the mechanism of frost injury have formed an interesting chapter in plant physiology, for it seems that the more commonly understood physical phenomena play an important rôle in determining the resistance of plants to frost. In the resistance of a plant to freezing the relative importance of such factors as undercooling of the tissue, the freezing point of the cell sap, and the precipitation of proteins is much disputed, owing probably to the fact that different plants have been investigated by the various authors.

In the investigation reported in this paper experiments were made to determine the physiological changes found to occur under this treatment in such plants as cabbage (*Brassica oleracea capitata*), and tomatoes (*Lycopersicon esculentum*).

The method of hardening commonly used is to expose the succulent plants in coldframes for a week or more to temperatures somewhat above the freezing point.

REVIEW OF LITERATURE

The mechanics of the process of ice formation within tissues have been investigated by Duhamel and Buffon (*9*).[2] They ascribed frost injury to rupture of the cells by growing ice crystals. Göppert (*12*) and Sachs (*42*, *43*), however, found that ice formation takes place mostly in the intercellular spaces, and hence rupture of the cells does not cause injury from freezing. Müller (*37*, *39*) reported that ice formation within the cell takes place only on rapid cooling and that ice formation within the tissue was necessary to produce a true frost injury. Wiegand (*48*, *49*) observed microscopically the point of first formation of ice crystals and their increase in size in the intercellular spaces.

[1] In the latter part of 1915 the work here presented was undertaken at the suggestion of Dr. R. H True, Physiologist in Charge of Plant Physiological Investigations, to whom the writer owes much for advice and direction. The writer is also indebted to Dr. William Crocker, Dr. S. H. Eckerson, Dr. F. C. Koch, and other members of the faculty of the Hull Biological Laboratories of the University of Chicago for instruction and for the use of equipment at that institution.

[2] Reference is made by number (italic) to "Literature cited," p. 108–111.

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Undercooling was found by Mez (*33*) to be a factor of great importance in frost injury. Where ice formation occurs at once, the lower tissues of trees are protected by the poorly conducting layers of frozen tissue on the outside. Voigtländer (*47*) showed that, owing to the continual movement of plants in the wind, but little undercooling of the cell sap occurs in nature.

A mechanical effect of ice formation upon the plasma membrane has been ascribed by Maximow (*30*, *31*, *32*) as a cause contributing to the frost injury of cells. He stated that the osmotic properties of the plasma membrane are changed by freezing, being supported in this opinion by Chandler (*5*).

Molisch (*36*) and Müller (*37*, *39*) ascribed frost injury to the withdrawal of water from the plasma membrane, and with this view Maximow partly agrees. The former authors did not follow the effect of desiccation farther than to state that the injury is due to the withdrawal of water during freezing. The processes of freezing, desiccation, and plasmolysis were found to be analagous in their effects upon the cell by Matruchot and Molliard (*28*, *29*). The analogy of these processes is further indicated by the work of Greely (*14*).

Gorke (*13*) advanced the idea that frost injury is due to the precipitation of proteins through salting out. He considered the concentration of the salts of the cell sap on freezing to be sufficient to precipitate irreversibly the proteins in solution in the cell sap and to cause similar changes in the protoplasmic gels. He considered the effect of the increased concentration of acid salts to be small, however, and insufficient to account for the precipitation. He was supported in this statement by later workers, including Schaffnit (*44*) and Lidforss (*23*). Voigtländer (*47*) doubted that the salting out of the proteins accounts for frost injury. Chandler (*5*) maintained that protein precipitation does not occur to any significant degree. He found that plants increase in hardiness when given nutrient salts in abundance, while, in accordance with Gorke's (*13*) theory, these plants should be more easily injured, owing to the increased salt content of their cell sap. It therefore appears that some factor other than salting out is necessary to account for the injury.

The accumulation of sugars and consequent increased depression of the freezing point of the cell sap was observed by Müller (*38*) in plants exposed to low temperatures. The effect of this increased content of sugars was followed by Lidforss (*23*), Schaffnit (*44*), Kovchoff (*21*) and Bartetzko (*1*). All of these authors agree in ascribing to sugars an important rôle in the prevention of protein precipitation owing to its protective effect for colloids.

The lesser injury from freezing shown by hardened plants was ascribed by Gorke (*13*), Schaffnit (*44*), and Bartetzko (*1*) to changes in the proteins. Schaffnit thought these changes consisted in a cleavage of the "high

molecular" forms of the proteins to simple forms, which are not so easily precipitated by the salting-out process.

In the plants which have been tested in this study all of these factors seem to play a part. In addition to these, there appears another important factor, which for the most part has been disregarded, the change in the actual acidity or hydrogen-ion concentration of the plant juice on freezing. It seems that this factor supplies the deficiencies of the other factors in explaining frost injury.

FIRST INDICATIONS OF FROST INJURY AND THEIR DEVELOPMENT IN CABBAGE AND TOMATO

The first indication of frost injury in the herbaceous plants which have been observed is in the appearance of injected areas. Where the exposure to low temperature has been of short duration, these areas appear as isolated dots over the surface of the leaf, as is well shown by cabbage and tomato (Pl. 7); and occasionally along the stem also, observed on sunflower (*Helianthus annuus*). The injected spots when observed by transmitted light are more transparent than the remainder of the leaf. The transparency is due to the displacement of air, which is ordinarily present within the intercellular spaces of the spongy parenchyma, by water which has been withdrawn from the cells during the process of freezing. It was shown by Sachs (*42*, *43*) that in the process of freezing, water passes out from the protoplast and freezes in the intercellular spaces. On thawing, this water is then left in the intercellular spaces until such time as it is evaporated or reabsorbed by the cells which have been plasmolyzed by freezing. Under certain conditions it requires considerable time for the protoplast to return to its former position against the cell wall and to regain its turgidity.

The tomato leaf does not survive ice formation in the tissue; consequently the injected spots appear as brown areas after a few hours. Sections of these spots made immediately after freezing show a collapsed condition of the palisade cells (Pl. 8, A). The collapse of the palisade causes depressions in the leaf surface, and these areas dry up after a few days. Death of the injured cells produces the spotted appearance shown in the leaf at the left of Plate 7, B. The cells around these areas, although exposed to the same temperature, show no injury because there was no ice formation. The effect of short exposures to low temperature appears to be nil, the injury being an accompaniment of ice formation. Voigtländer (*47*) has previously shown that there must be ice formation in the tissues to produce frost injury.

MECHANISM OF FREEZING

The undercooling of the cell solution is a factor of great importance in the resistance of cabbage to freezing. Those plants which have the most bloom on the leaf surface are most resistant to the formation of

ice within the tissue. Cabbages which are well covered with wax show no indications of freezing after several hours' exposure to a temperature 5 degrees below that at which ordinary plants show ice formation.

When one considers the physical mechanism of freezing in the leaf, the cause of the resistance is found to be this coating of wax. In the natural state moisture is usually present on the surface of leaves, even though in minute quantities. This may be deposited by condensation from the surrounding atmosphere. In cooling down to only slightly below zero this water freezes, for the plant is seldom motionless enough to allow much undercooling, as shown by Voigtländer (*47*). In plants which have but little wax greater amounts of moisture stick to the leaves, while those covered with wax are not wet. This can be observed if such leaves are immersed in water. Those with a thick coating of wax have a bright silvery sheen, owing to the lack of wetting of the leaf surface. If the wax is rubbed off, the surface then becomes wet and loses its ability to form a mirror surface.

Water which freezes on the leaf surface serves to inoculate the undercooled solution within the leaf; in fact, the injected spots observed are caused by this inoculation. When once begun, the freezing process is transmitted rapidly through the undercooled leaf tissue, and the frozen spot enlarges until the whole leaf may be frozen. That water on the surface may cause the inoculation is easily shown by placing a drop of water on the leaf and exposing it to $-3°$ C., when the area beneath the drop will be found to freeze first and show injection. Inoculation probably takes place through stomata or through cracks in the wax and will take place less frequently if the wax is thick.

Bigelow and Rykenboer (*2*) have recently shown that a very great undercooling can occur in capillary tubes. Stomata and cracks in the waxy covering of the leaf are of small enough dimensions to allow considerable undercooling without ice formation taking place through them to inoculate the tissue beneath. It would appear consequently that where inoculation occurs the openings are largest.

Freezing in spots is of common occurrence in plants. It has been observed to occur on cabbages when they are exposed to frost in the open. The injected areas have been produced on various greenhouse plants, including begonia, salvia, geranium, coleus, bryophyllum, lettuce, sunflower, hydrangea, and *Aucuba japonica*.

Unhardened cabbage leaves survive ice formation within the tissue when the injected area does not cover too great a part of the surface. Only slight plasmolysis can be observed in sections of the injected spots in this case. On fixing with acid alcohol (Carnoy's solution) cabbage leaves which have been frozen in spots, large masses of spherocrystals were found, quite sharply limited to the injected areas and often not to be found in the rest of the tissue. These crystals (Pl. 8, B) appear to be

calcium malophosphate. Evidence of the identity of this substance is given by the spherocrystalline form (*46*); its reaction with ammonium molybdate and magnesia mixture, showing the presence of phosphates; replacement of the spherocrystals by gypsum crystals on treatment with dilute sulphuric acid; carbonization on treatment with concentrated sulphuric acid; slow solubility in water; solubility in saturated solutions of dicalcium and tricalcium phosphate and insolubility in saturated monocalcium phosphate. Crystals of maleic acid were obtained by microsublimation from cabbage leaf. After fixing in Carnoy's solution these crystal patches are to be found in great abundance in the older leaves, which are not frozen as easily as the leaves up to an inch in length. The crystals are more abundant in hardened than in nonhardened cabbage leaves of the same size. Precipitation of the malophosphate in the injected areas seems to be caused by greater concentration there, owing to some effect of the freezing. Since Carnoy's solution kills the tissues very rapidly, it is not probable that a diffusion from the surrounding tissue into these spots would occur to any great extent.

GROWTH DEVELOPMENT IN THE INJECTED AREAS

On standing at room temperature for a few minutes the injected areas of cabbage often disappear, and no trace of them can be seen for two or three days after freezing. At about the third day the spots again become evident as slightly raised areas sharply defined. The raised portions are a little lighter in color than the rest of the leaf. A decrease in the number of chloroplasts in these areas gives the leaf a mottled appearance. Similar conditions have been observed by Ritzema Bos (*41*) on other plants of this genus (*Brassica napus*) as a result of frost injury. The young intumescences grow very rapidly for 10 days or more and may reach a relatively enormous size, showing in section a thickness many times that of the normal leaf. They are often of a circular shape, but they may have any shape, corresponding to the coalescence of the injected areas as they increase in size (Pl. 9). When a large portion of the tissue is injected, it is difficult to keep the entire leaf from dying, but death may be prevented by placing the plant in a saturated atmosphere. The entire leaf may be a mass of intumescences so that it is rolled and thickened in all manner of shapes. The swellings occur along the veins of the leaf more abundantly than over the remainder of the leaf surface (Pl. 9, B), although the portions about the veins seem no more liable to injection than other parts.

In section the tumors when about 4 days old are seen to consist of enormous cells with large nuclei (Pl. 8, C). These large cells are often bi- or tri-nucleate, a condition commonly observed in pathological conditions. The walls of these cells are quite thin, and large vacuoles appear in the protoplast. The peculiar large cells recall the pathological

condition of cells recently found by Samuels [1] to accompany the process of precipitation of raphides of calcium oxalate.

Quite often in older intumescences there seems to be a return to the meristematic condition in certain cells, so that a cambium-like layer is formed which pushes out conical-shaped rows of cells to form the tumor (Pl. 10, A). After two weeks' growth the tumors begin to die back, and infection may occur (Pl. 10, B). Before this time no bacteria have been observed in the formation of the tumor.

Growth is sharply confined to the area injected and does not spread to the other cells. Such limitation would hardly be the case if bacteria were concerned in the growth stimulus. The growth is not due to any condition peculiar to the cabbages used, for identical growths have been obtained during different years. Similar intumescences have been produced from frost-injured spots on the leaves of *Bryophyllum calicynum*.

Small growths have also been observed to be produced from injected areas on the leaves of lettuce and salvia. The cells of injected areas of hardened cabbage leaves are not stimulated to growth by moderate freezing. Since plasmolysis of the cells occurs in this case, as well as in the cells of nonhardened plants, it appears that plasmolysis alone is not the cause of the growth stimulus.

Young intumescences contain large quantities of dextrose, much more than the ordinary leaf cells. Levulose is present in only small quantities, if at all. When heated with Fehling's solution, an abundant precipitate of cuprous oxid is formed in the tumors, giving them a reddish color. Starch and tannins are absent from the hypertrophied areas.

PEROXIDASE CHANGES IN THE INJECTED AREAS OF CABBAGE LEAVES

A lot of cabbage leaves were spotted and placed in the greenhouse to allow the development of tumors. From time to time samples of the spotted leaves were tested for peroxidase. Tetramethylparaphenylenediamin in 60 per cent alcohol was used as the reagent for oxidase, and 10 per cent of commercial hydrogen peroxid was added to this to demonstrate the peroxidases. In these reagents the activity of the solutions was tested on sections of potatoes on which known reactions were given. Little or no reaction for oxidase was obtained in cabbage leaves within the time in which potato sections gave a good reaction. This was supported by testing the leaf juices in the Bunzell (*4*) apparatus, which showed comparatively little oxidase activity with tyrosin, pyrogallol, hydroquinone, and pyrocatechin. It was found that with tetramethylparaphenylenediamin a good test for peroxidase was given along the veins of the cabbage leaf. In leaves tested immediately after spotting no more peroxidase reaction was given in the injected than in other areas; nor could any greater amount of peroxidase be demonstrated in

[1] Paper read before the American Association for the Advancement of Science, New York, 1916.

the spots until the growth of the intumescences began. At about the second or third day after freezing, the spotted areas showed more or less irregularly an increase in the peroxidase reaction. In young tumors which were growing rapidly there was a marked increase in the peroxidase reaction of the tumors over the rest of the leaf, with the exception of the veins. Plate A gives an idea of the relative peroxidase reaction shown by the purple color in the tumors as well as the relative color of tumor and normal areas of the leaf before applying the reaction. In all cases leaves which were plunged into boiling water for two minutes gave negative tests for peroxidase.

Woods (*50*) found greater amounts of peroxidase in the spotted areas of tobacco leaves infected with mosaic than in the normal areas of the leaf. He attributed the lighter color to the oxidation of the chlorophyll by the abnormally increased peroxidase. He submitted evidence to show that chlorophyll is destroyed by peroxidases *in vitro*. It is possible that there is a similar connection in the case of tumored cabbage leaves and that this accounts for the lighter color of the intumescences.

These abnormal growths were first obtained in the winter of 1915 in great abundance and at will. The only conditions necessary are to have the temperature to which the cabbages are exposed so regulated that the freezing can be stopped before the leaf is killed by being frozen throughout. The temperature required for cabbages is about $-3°C.$, with an exposure of about 30 minutes. In June, 1916, Smith (*45*) began some experiments on the production of abnormal growths by injecting certain simple chemical substances or exposing plants to the vapor of these substances. This interesting work has already been reported. The chief point in his report to be noticed here is that a number of these substances, ammonia, acetic acid, amins, etc., are either acid or alkaline in reaction, and some strongly so, a point which will be discussed later. Among other plants tested, cauliflower plants (*Brassica oleracea botrytis*) were exposed to ammonia vapor. The intumescences which resulted might well be taken for those shown in the illustrations in this paper, so similar are they. The differences seem to be that on exposure to ammonia a smaller area is affected, such as that about a stomatal opening. In Plate 63 of Smith's report (*45*) the growths appear along the veins in abundance. A similar stimulus can be given by freezing, a purely physical process at the start, as well as by chemical treatment, indicating a similar basic cause in the two processes.

Intumescences of this kind are quite commonly found on cabbage, resulting from aphis punctures and from breaking the leaf surface; injections of water which loosen the epidermal layer produce them; in fact, it is only necessary to puncture the epidermis to cause their development. Hence, it seems that the growth response is a common phenomenon in cabbage and that it may be caused by a condition common either to

exposure to acid or alkaline vapors, to freezing, or to drying by excessive transpiration from exposed areas. The factor active here seems to the writer to be a change in the reaction of the plant juice, accompanied also by an increase in the concentration of the salts of the cell sap. That a change of acidity might occur from exposure to ammonia or acetic-acid vapor is not difficult to see. The change in reaction of the plant juice on concentration by transpiration is shown by the following experiment:

Fresh cabbage leaves were taken and divided into two lots. The first half was kept in a cool, moist atmosphere; the other half was allowed to wilt in a dry room until turgidity had been lost. Then both samples were ground in a meat chopper, and the juice was expressed. The acidity of the leaf juice was increased by the wilting from 2.05×10^{-6} to 5.37×10^{-6} H^+. Evidence of the change of reaction on freezing will be given later.

By placing plants which have been injected in spots into tap water for from 5 to 12 hours the formation of tumors can be stopped. This inhibition was accomplished by inverting a potted cabbage over a beaker of tap water so that all or part of the leaves were immersed. The coating of wax on the leaves prevents their wetting by the water and holds a layer of air about the leaf. This treatment effectually stops transpiration, although it may also produce abnormal conditions, such as a poor oxygen supply. When treated in this manner, the injected areas fail to develop into tumors, and this hindrance is confined to those spots which are immersed. It is interesting in this regard to note the old observation of Sachs (*42*, *43*) that slow thawing, as he regarded it, decreased frost injury. His method was to place the frozen tissue into water and allow it to thaw out slowly. Müller (*37*, *39*) showed that this process in fact caused a very rapid thawing, owing to the rapid thermal exchange. Hence, the lessened injury must rest on some basis other than slow thawing of the tissue. Greenhouse men have generally observed that frost injury is much less if the frosted plants are at once sprinkled thoroughly.

PLASMOLYTIC BEHAVIOR OF TUMOR CELLS

The cells of young cabbage tumors are plasmolyzed at lower concentrations and in shorter time than the mesophyll cells in adjoining normal tissue. Sections were made from fresh tumors, thin enough to be observed by the low power of the microscope and still thick enough to leave several uninjured cells. It was found to give greatest regularity if the tumor cells were compared with the normal mesophyll cells from the same section for their plasmolytic behavior. Observations on the plasmolytic limits of such tissues are very difficult and are mainly comparative rather than to be taken as a measure of actual osmotic pressure or permeability.

Table I shows the plasmolytic limit concentrations after 30 minutes' exposure.

TABLE I.—*Plasmolytic limit concentrations for normal and tumor cells*

Kind of cell.	Freezing points of solutions.			
	Glycerin.	Sucrose.	Potassium nitrate.	Calcium nitrate.
	°C.	°C.	°C.	°C.
Normal mesophyll	−1. 943	−3. 290	−1. 733	−1. 462
Tumor cells	−1. 132	−1. 509	−1. 461	− . 952

These values are for young tumors about 5 days old and in active growth. The age of the tumor has much to do with its plasmolytic behavior. Very old tumor cells which have stopped growth show no difference in plasmolytic behavior from normal mesophyll cells of the same leaf. The writer is inclined to regard the difference in the plasmolytic behavior as being due, not to a difference in the osmotic concentration of the cell sap, but rather to differences in permeability, especially to water, and probably also to the substance used for plasmolysis. Smith (*45, p. 184*) says:

> . . . it would seem . . . that in local osmotic action (possibly in some stages chemical action also) of various substances . . . we have . . . the explanation of tumor growth. . . .

This statement evidently implies a changed permeability as well as pure osmotic effect. The writer has determined the freezing points of juices expressed from cultures of tumors caused by *Bacillus tumefaciens* grown on daisy from Smith's cultures, and has found a lower freezing point for tumor tissue than for either stem or leaf tissue. Evidence offered by the greater freezing-point depression of saps of parasites than of saps from the host (*17*) seem to indicate that such an excess depression is a common condition for parasitic tissues (*24*). The accumulation of such large quantities of sugar in the tumors of cabbage caused by freezing leads the author to believe that there is some change in the cells which allows them to obtain it from their neighbors in the surrounding areas. The quantities of starch present in the cells before freezing are not sufficient to account for this much sugar, for the young leaves in which tumors develop rapidly contain very little starch. The decrease in chlorophyll would hardly be in accord with the synthesis of the sugar within the tumor cells.

It is probable that in the frozen cells of the injected areas there occur changes in the state of the protoplasm constitutents so that more sugar is adsorbed there, or so that the sugar is held there in some sort of chemical combination. Osterhout (*40*) suggests that differences in

permeability alone do not effect the accumulation of substances within cells.

So far the writer has not obtained the freezing point of juice expressed from cabbage tumors caused by frost injury. However, some evidence of the relative freezing points of the tumor cells and leaf cells can be gained from the following experiment: Cabbage plants showing an abundance of young tumors were placed at a constant temperature just sufficiently low to freeze the tissue. It was observed that on freezing the leaves were frozen in spots over the areas not covered by tumors usually before the tumor cells were frozen. One would expect the tumors to freeze much more quickly than the normal tissue if the relative osmotic concentrations were those shown by plasmolysis. The failure of the tumors to freeze can not be attributed to greater undercooling in them, for the cells have thin walls and are not covered by wax.

PHYSIOLOGICAL CHANGE IN CABBAGES DURING THE HARDENING PROCESS

The physical changes in cabbage which have been observed during the hardening process are a slowing of the growth rate so that the plants are smaller than those of the same age grown at a warm temperature and consequently are more mature; and an increase in the amount of bloom on the leaves. The condition of hardiness can well be judged by the stiff, springy condition of the leaves. Hardened leaves are 20 per cent thicker than nonhardened leaves of the same age.

By exposure to temperatures a few degrees above the freezing point for a week or so the Early Jersey Wakefield cabbage acquires the ability to be frozen stiff without injury, or with only slight injury (Pl. 11, A). Plants frozen directly after being taken from the greenhouse are either injured in spots or killed throughout, according to the extent of the frozen area and the temperature. Tomatoes under similar hardening treatment can be made to withstand somewhat lower temperatures without freezing; but once the hardened plants become frozen they are killed (Pl. 11, B). Tomatoes are killed or injured on long exposure to a temperature of 5° C. This injuring is not a true freezing to death, according to Müller (*37*, *39*), for no ice formation takes place in the tissue. Molisch (*36*) explains it on the basis of accumulation of toxic substances through poor oxidation. Tomatoes which can not survive ice formation within the tissue can hardly be said to show a true hardiness to frost. The term "hardiness" should be applied to the ability of a plant to survive ice formation within its tissues, as shown by cabbage.

A condition of greater resistance to freezing can be produced in cabbages by watering them with solutions which check growth. Plants watered with nutrient solutions which produce rapid growth, such as *N/10* potassium nitrate, *N/10* calcium nitrate, or Knop's 2 per cent, are

much more easily injured than those watered with *N/10* sodium chlorid or *N/10*, sodium bicarbonate. The latter solutions, like exposure to low temperature, retard growth. Cultures watered with all the foregoing *N/10* solutions showed a lower freezing point than controls grown in poor clay soil. An increase in the cell-sap concentration and lowering of the freezing point can also be produced by growing plants in dry soil. The increased resistance of cabbage watered with salt solutions indicates that it is not the increase of concentration of just the salts of the cell sap on freezing that causes injury.

METHOD OF TESTING HARDINESS

An electrically-controlled constant-temperature room which can be maintained at any desired temperature within a few tenths of a degree was used in determining the hardiness of the plants under test. This compartment was cooled by the direct expansion of ammonia. The temperature was kept the same throughout by keeping the air stirred vigorously with an electric fan. Plants were exposed to a temperature just sufficient to freeze them, and they were then observed at intervals for the appearance of injury. Comparative tests were run together and for the same length of time.

TIME AND TEMPERATURE FACTORS IN ACQUIRING OR LOSING HARDINESS

The time necessary for plants to become hardened was determined by placing cabbage in dark chambers at constant temperatures of 3°C., and 5°, using 18° and 25° as controls. Exposure for 24 hours to 3° was found to produce a slightly increased hardiness as judged by the extent of the injury. After 5 days' exposure to 3° the cabbages were not injured by 30 minutes' exposure to −3°, although frozen stiff. The control plants were killed throughout. On placing such hardened plants in the greenhouse at room temperature and at a constant temperature of 18° in the dark, it was found that the hardiness was lost in the greenhouse in about the time taken to acquire it, while the hardiness lasted a few days longer at 18°. By alternating cabbage plants between 3° and 25° it was shown that the hardening process is an accommodation brought about not by changes of temperature such as occur in the natural hardening of plants, but by low temperature. From the above statements it is seen that plants acquire and lose hardiness rather rapidly. Under natural conditions the hardiness acquired in one night of low temperature may be lost during the succeeding warm day, and there is accumulative effect only when the average temperature is low.

The maturity of the tissue is a factor of great importance in frost resistance. Young leaves of cabbage are more easily injured than old leaves. During hardening these young leaves become resistant, indi-

cating that they rapidly pass through some sort of maturation process. The more common injury to the cells about the veins indicates that there is a physiological difference between the vascular tissue and the other cells of the leaf. This physiological difference is suggested also by Mangham (*27*) in a recent article.

FREEZING POINT AND EXPRESSION OF CABBAGE JUICES

A large number of determinations were made of the freezing points of juice expressed by different methods from hardened and nonhardened plants. As Dixon and Atkins (*8*) have shown, the method of treatment before expression has a considerable influence on the depression values. However, there is about the same difference between the freezing points of juices expressed from hardened and nonhardened cabbages regardless of the method of treatment before expression. Tests were made after freezing in liquid air, after freezing with solid carbon dioxid, after freezing at −5° C., and without freezing. One of the most convenient methods is to freeze the tissue with carbon dioxid until brittle and then to grind it in a mortar to a fine powder. The material can then be transferred to the press and allowed to thaw out. This method is more available than the liquid-air method and is less expensive. The sap was expressed either in a large hand press which left a marc practically dry or in a hydraulic press under a pressure of 10 to 30 tons on a 2½-inch ram. Table II gives the comparative values.

TABLE II.—*Depression values for cabbage juice*

Condition.	Not frozen.	Method of freezing before expression.		
		−5 °C.	Carbon dioxid.	Liquid air.
	°C.	°C.	°C.	°C.
Hardened	−0.985	−1.160	−1.630	−1.822
Nonhardened	−0.910	−1.122	−1.530	−1.668
Excess depression of hardened over nonhardened	−0.076	−0.036	−0.080	−0.154

Average excess depression of hardened over nonhardened, 0.085°C.

The smallest difference, 0.036° C., shown after freezing at −5° may be due to a greater injury to the nonhardened leaves and corresponding increase in concentration of the expressed juice. The increase of the freezing-point depression on treatment with solid carbon dioxid and liquid air over that given by freezing at −5° may be due to changes in the cell membranes which make them more permeable to osmotically active substances. The process of freezing, especially to this degree, is no innocent procedure and may result in changes which produce protein precipitation. Consequently, juices expressed in this manner do not represent the true concentration of the cell sap for all its constituents.

It is quite clear that the difference of about 0.1 degree in the freezing point of the cell sap from hardened and nonhardened cabbages is insufficient to account for the resistance to low temperature shown by hardened plants, for hardened plants are not injured by being frozen at a temperature 3 degrees below the killing temperature for nonhardened plants (*6*). Since ice formation takes place in this case, the freezing point of the cell sap must be exceeded.

CHEMICAL CHANGES DURING HARDENING

CARBOHYDRATES.—In studying the carbohydrate changes during the hardening process cabbage plants from the same lot were placed part in the greenhouse and part in a coldframe. The plants were allowed to harden in the coldframe until they were not injured by being frozen stiff at −3° C. Three samples of leaves, 25 gm. each, were taken from each lot for starch and sugar determinations. The percentage values are shown in Table III.

TABLE III.—*Analyses of carbohydrates in cabbage* [a]

Sample No.	Reducing sugars as glucose.	Dissaccharids as sucrose.	Polysaccharids as starch.
Hardened cabbages:	*Per cent.*	*Per cent.*	*Per cent.*
A1	3.13	1.91	2.69
A2	3.03	2.05	2.75
A3	2.56	2.33	2.45
Average	2.91	2.10	2.63
Nonhardened cabbages:			
B1	1.66	.21	3.36
B2	1.54	.19	3.39
B3	1.48	.18	3.41
Average	1.56	.19	3.39

[a] The writer is indebted to Mr. J. W. Kelly, of the Office of Drug-Plant, Poisonous Plant, Physiological and Fermentation Investigations, for the carbohydrate determinations.

In the average of these values it is seen that the equilibrium between starch, glucose, and sucrose in the nonhardened plants in which polysaccharids predominate is displaced in hardened cabbages in the direction of the mono- and di-saccharids. Lidforss (*23*) found this to be a common transformation in plants during the cold season. Hasselbring and Hawkins (*18*) have found a similar condition to occur in sweet potatoes kept at low temperatures. They submit evidence to show that the increase in sucrose takes place only after glucose formation from the starch. It is difficult to maintain cabbage plants without photosynthesis for a sufficient time to observe if that is the case here.

The writer has been unable to find any considerable amount of levulose in cabbage leaves by microchemical means.

The cleavage of polysaccharids yields osmotically active substances which account for the lower freezing point of juices expressed from hardened plants. As Schaffnit (*44*) has shown, the sugars produced may have a considerable influence on frost resistance by allowing the solution to be undercooled to a greater degree or by preventing the precipitation of proteins.

The carbohydrate changes were determined also in plants kept at constant temperatures in the dark to determine the relation of carbohydrate change to the time of acquiring hardiness (Table IV).

TABLE IV.—*Analyses of carbohydrates in cabbage plants kept at constant temperatures in the dark*

Temperature.	Duration.	Starch.	Reducing sugars.	Sucrose.	Freezing point of sap.	Condition of hardiness.
°C.	*Days.*	*Per cent.*	*Per cent.*	*Per cent.*	°C.	
Greenhouse	.....	1.72	0.70	0.076	−0.816	At −2.5° C. completely killed in in 60 minutes.
18	5	1.72	1.00	.00	− .675	At −2.5° C. two-thirds killed in 60 minutes.
5	5	1.64	1.09	.066	− .725	At −2.5° C. not injured in 60 minutes. Frozen stiff.
18	10	1.19	.48	.16	.....	At −4.5° C. all killed in 60 minutes.
5	10	1.33	.98	.015	.....	At −4.5° C. one-third killed in 60 minutes. Frozen stiff.
Greenhouse	.....	1.37	1.17	.06	− .780	At −3° C. all killed in 30 minutes.
25	6	1.33	.76	.15	− .746	At −3° C. all killed in 30 minutes.
3	6	1.19	.72	.19	− .797	At −3° C. not injured in 30 minutes. Frozen stiff.
25	10	1.00	.16	.05	.....	
3	10	1.30	.80	.15	.....	

From Tables III and IV it is seen that hardiness is acquired before any great change occurs in the carbohydrate equilibrium. A utilization of both starch and the reducing sugars in cultures kept at higher temperatures is noticeable, as well as the lack of any great change at the lower temperatures.

Analyses of hardened and nonhardened cabbages were also made by a modification of Koch's (*20*) method. This method involves a separation

into a lipoid fraction (F_1), a water-soluble fraction (F_2), and a fraction insoluble in alcohol and water (F_3). Samples of 100 gm., green weight, were collected from hardened (H_1, H_2) and nonhardened (NH_1, NH_2) cabbages and preserved in 85 per cent alcohol with the addition of 0.5 gm. of calcium carbonate. The weights given in grams in Table V express percentages on the basis of green weight.

TABLE V.—*Complete analyses of hardened and nonhardened cabbages*

Constituent.	H_1	H_2	NH_1	NH_2
Total solids	10.1844	10.5604	9.035	9.2945
Moisture	89.8186	89.4396	90.965	90.965
Total phosphorus	.0669	.0664	.0535	.0537
Total nitrogen	.3015	.3024	.2986	.2915
Total lipoid phosphorus	.0124	.0121	.0089	.0093
Total lipoid nitrogen	.0112	.0129	.0146	.0123
Organic solids F_2	1.5037	1.6137	1.0859	1.2750
Phosphorus F_2	.0023	.....	.0029	.0041
Nitrogen F_2	.....	.0445	.0459	.0464
Ammonia F_2	.0010	.....	.0007	.....
Total Van Slyke nitrogen	.0168	.0162	.0084	.0068
Organic solids	5.8603	6.0869	4.8756	5.0593
Phosphorus F_3	.0522	.0520	.0417	.0403
Nitrogen F_3	.....	.2458	.2381	.2328
Water-soluble phosphorus F_3	.....	.0314	.0265	.....

The percentage of moisture in hardened cabbages is decreased slightly with a corresponding increase in the organic solids of the third fraction (F_3). It is difficult to obtain the same degree of humidity under such different conditions as used for hardening, and this may well account for a difference of 1 per cent of moisture, as shown here. In the hardened plants the increase in total nitrogen and phosphorus is noticeable. The increase in phosphorus is in accord with the finding of greater amounts of calcium malophosphate in hardened plants by microchemical means.

The increase in the aminonitrogen of hardened plants is suggestive of the changes which occur in the proteins. Schaffnit (*44*) found the proteins from hardened plants to be more difficult to precipitate by freezing than those from greenhouse plants. He ascribed this to changes in the proteins resulting in the cleavage of the more labile "high molecular" forms. According to the above analyses, the aminonitrogen represents 35 per cent of the total nitrogen of the second fraction (F_2) in the hardened cabbages and about half that amount in the nonhardened plants, or 5.5 per cent of the total nitrogen in the first case and 2.5 per cent in the second case. Although this is a small change, it is not necessary nor probable that cleavage as far as to the amino acids should occur to prevent precipitation on freezing.

CHANGE OF HYDROGEN-ION CONCENTRATION OF A PLANT SAP ON FREEZING

INDICATION OF A CHANGE OF ACIDITY AS SHOWN BY COLOR CHANGES

One of the most striking of the changes which occur as a result of freezing is a change of color in plants which have colored leaves. Haas (*15*) has shown that the plant pigments act as an indicator of the cell-sap acidity. The change of reaction is best observed in such plants as coleus, which have the pigment in solution in the cell sap and not masked by chlorophyll. Using such a natural indicator is advantageous because it introduces no external factors. Besides, the range of hydrogen-ion concentration covered by the change of the pigment from red to blue is the same as that naturally occurring in the plant. Everyone has observed that various conditions affect the color of such pigments.

The color change on freezing is due to a change in the acidity of the plant juice. The juice of the variety of coleus used here reacts slightly acid to the pigment, as shown by the bright-red color. If coleus leaves are frozen, the reaction remains the same for a considerable time. If, now, the leaf is rapidly thawed out by being dipped into warm water, the red pigment at once changes to a decided blue color. This change can be observed almost in an instant, before oxidase activity can produce secondary changes. The rapidity of the change indicates a removal of the excess hydrogen-ion in much the same manner as by neutralization.

Similar changes of reaction can be produced in the white of egg or in cabbage juice on freezing. If methyl red is added to cabbage juice and the juice allowed to freeze, an increase in the acidity is indicated by the increasing red color of the indicator. Here the change is more or less masked by the chlorophyll. A similar change of acidity can be observed if the white of egg is frozen. It is necessary to use an indicator covering the proper range of acidity, in this case phenolphthalein, since white of egg is slightly alkaline. The white of egg is colored red by the phenolphthalein; but when frozen solid, the red color disappears, owing to an increase in the hydrogen-ion concentration. This latter case is to serve merely as a demonstration, since we are not especially concerned with such materials. The change of reaction here may be due to the high eutectic point of sodium bicarbonate and its separation in the solid phase on freezing. Certainly such changes in acidity as here shown have some influence on the precipitation of proteins and upon frost injury. It is well known from the work of numerous authors that the acidity of the medium is a factor of great importance in determining the state of hydrophilous colloids such as the proteins.

CHANGES OF REACTION ON PLASMOLYSIS

A change in reaction can be produced also by placing sections of coleus leaves in concentrated sugar solutions. The protoplasts of the trichomes are plasmolyzed, and the red color is deepened by the removal of water. After a time the color changes to blue, and at the same time the pigment begins to lake from the cell, indicating a change of permeability and the death of the tissue. If the plasmolysis is not carried too far nor allowed to continue until a change of color occurs, the cells regain their turgidity, and the pigment does not leach out. Hence, the duration of the reaction appears to be a factor in producing the injury, as well as the amount of change of acidity.

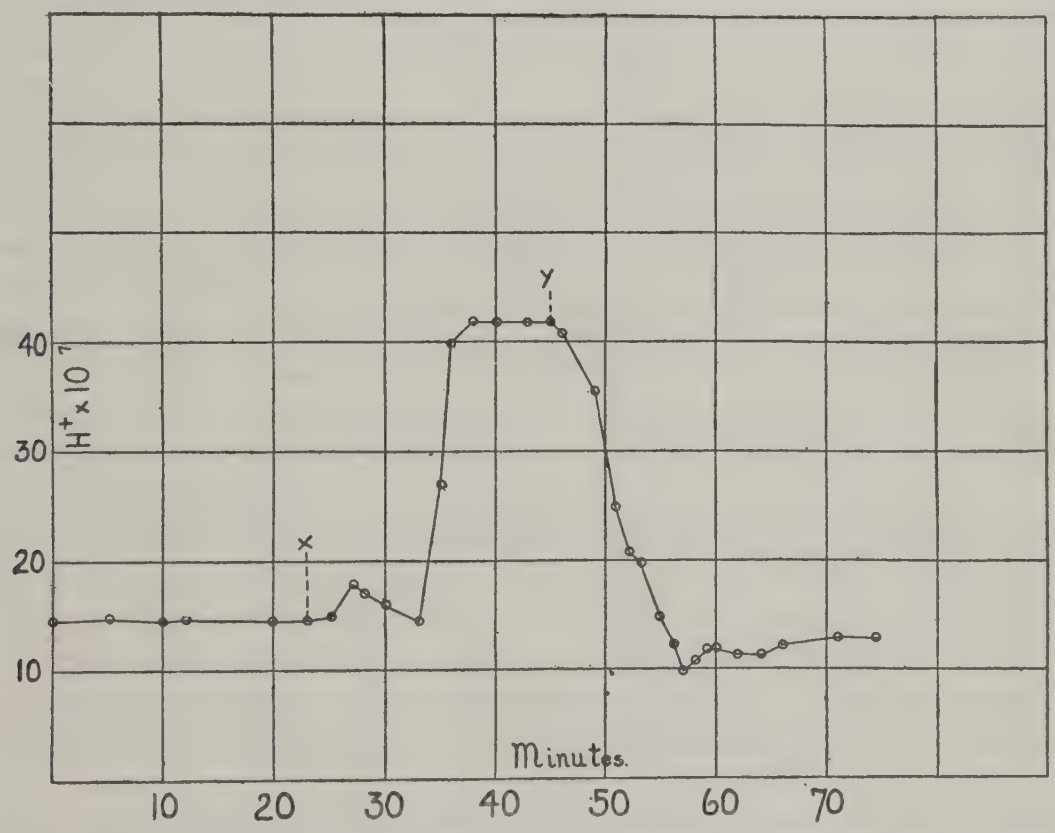

FIG. 1.—Graph showing change of the hydrogen-ion concentration of cabbage-leaf juice on freezing. Freezing at point *x*, thawing at point *y*.

POTENTIOMETRIC DETERMINATION OF THE CHANGES IN HYDROGEN-ION CONCENTRATION ON FREEZING

The change in acidity of a plant juice during freezing can be followed by the use of potentiometric methods. The potentiometer arrangement of Michaelis (*34*) was used. The measurements were made in a closed vessel provided with a dip hydrogen electrode essentially like that of Bovie (*3*) except that an exit tube was provided for the hydrogen.

As shown by the graph (fig. 1), the acidity of cabbage juice is increased by freezing. There is first a rise in the hydrogen-ion concentration, then a return to its original value, and then a very rapid rise to an acidity

much above that at the start. This value remains constant at the lower temperature and is directly proportional to the lowering of the freezing point. On thawing there is then a decrease in the hydrogen-ion concentration to a value less than the original, and then a return to the original value, or a value somewhat lower than the original. This change is almost paralleled by the increased acidity of a dilute solution of acid calcium phosphate, $Ca(H_2PO_4)_2$, on freezing. The graph for the plant juice differs from the latter, however, in the return to the original value at the start of the curve and in the depression past the original value on warming. This lag in the curve for the plant juice may be caused by removal of part of the hydrogen ions by proteins on freezing and the breaking of the combination on thawing out. If the juice is kept in the frozen condition for a longer time the final acidity is less than the original, evidently on account of some permanent combination of the excess hydrogen ion. This irreversible combination is indicated also by the color changes in coleus leaves.

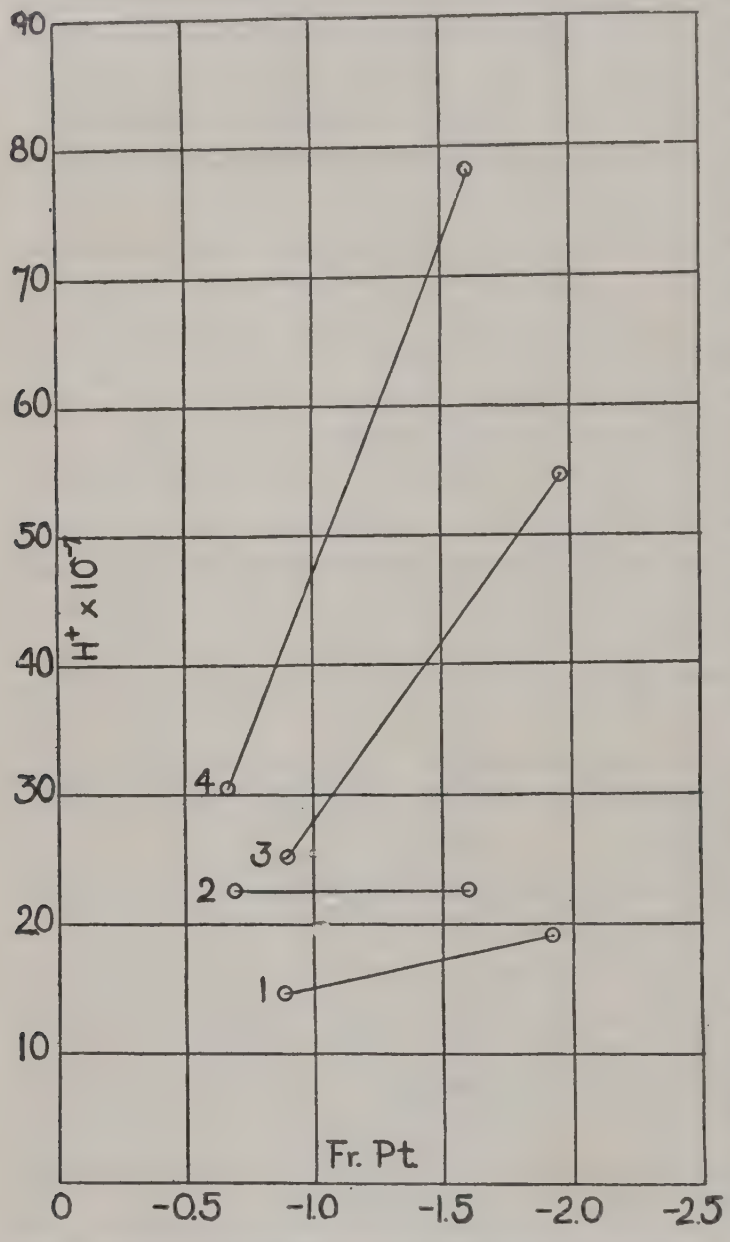

FIG. 2.—Graph showing increase in acidity with increasing depression of the freezing point on concentration of cabbage juice. 1 and 2, midrib juice: 3 and 4, iuice from leaf minus midrib.

There are many objections to this method of measuring the increase of hydrogen-ion concentration on freezing, such as the melting of the ice by the stream of hydrogen, and the lack of uniformity in distribution of the unfrozen juice. To be free from these hindrances the following procedure was adopted:

The midribs and petioles of fresh cabbage leaves were cut out. This separation gave a more or less quantitative division of the tissue, although a large amount of vascular tissue was still left in the leaf. Juice was obtained from these portions by grinding them in a meat chopper and then expressing. Samples of a large quantity of juice were then taken for the determination of the freezing point and the acidity. The remainder was frozen, and the concentrated juice expressed from the ice. This concentrated juice was then sampled for freezing-point determinations and for acidity. The graphs in figure 2 show on the y-axis the increase in acidity on concentrating by the amount shown by the depression of the freezing point given on the x-axis. Graphs 3 and 4 represent the changes of acidity of juices expressed from cabbage leaves with the midrib removed. Graphs 1 and 2 give the acidity changes for juices expressed from the midrib and petiole alone. The original acidity of the juice from the midrib is a little less than that for the juice from the rest of the leaf, although the freezing point is practically the same. On concentrating, however, the acidity of the midrib juice does not increase nearly so rapidly as that of the juice from nonvascular tissue.

The concentrated juices were rediluted by adding the ice from which they had been expressed and then allowing the mixture to thaw. There is, of course, some loss in expressing, but this is comparatively small, as shown by the freezing point values. Petiole juice having a freezing point of $-0.88°$ C. and an acidity of $1.46\times10^{-6}H^{+}$ was concentrated to a freezing point of $-1.94°$ and an acidity of $1.92\times10^{-6}H^{+}$. All of the ice removed was saved and then remixed with the concentrated juice, giving the rediluted juice a freezing point of $-0.85°$ C. and an acidity of $0.96\times10^{-6}H^{+}$. In a similar manner the values for juice from the rest of the leaf were originally: Freezing point $-0.90°$, acidity $2.5\times10^{-6}H^{+}$; after concentration, freezing point $-1.96°$ C., acidity $5.45\times10^{-6}H^{+}$; and on rediluting, freezing point $-0.85°$ C., acidity $2.33\times10^{-6}H^{+}$. The concentration was the same for both samples as measured by the freezing point; still there was a much less change in the acidity of the juice from the petiole than from the nonvascular tissue. On rediluting, the acidity of the petiole juice is less than its original value, while that for the rest of the leaf is practically the same. The same amounts of acid were added to the same volume of juices expressed from the midrib and from the rest of the leaf, and then the vials were placed on ice. The petiole juice precipitated much more quickly than the other sample.

BUFFER EFFECT OF JUICE FROM MIDRIB AND REST OF LEAF

The juice from the petiole and midrib of cabbage has a lesser buffer effect than that from the rest of the leaf. This is shown on titration with *N/10* sulphuric acid and *N/10* sodium hydroxid (fig. 3). The number

of cubic centimeters of $N/10$ acid or $N/10$ alkali is plotted on the x-axis and C_H on the y-axis. The graph for midrib juice has a much steeper slope than the graph for juice expressed from the rest of the leaf, indicating a lesser ability to combine with the acid or alkali added. Precipitation was observed at the points marked x. This shows the point of precipitation on neutralization of the free hydrogen ion of both samples to be about $10^{-6.57}$ C_H. On adding acid the albumens of the petiole juice are precipated at a lesser hydrogen-ion concentration than that for juice of the rest of the leaf. To reach the higher hydrogen-ion concentration a considerably greater amount of acid is required. For instance, 50 cc. of

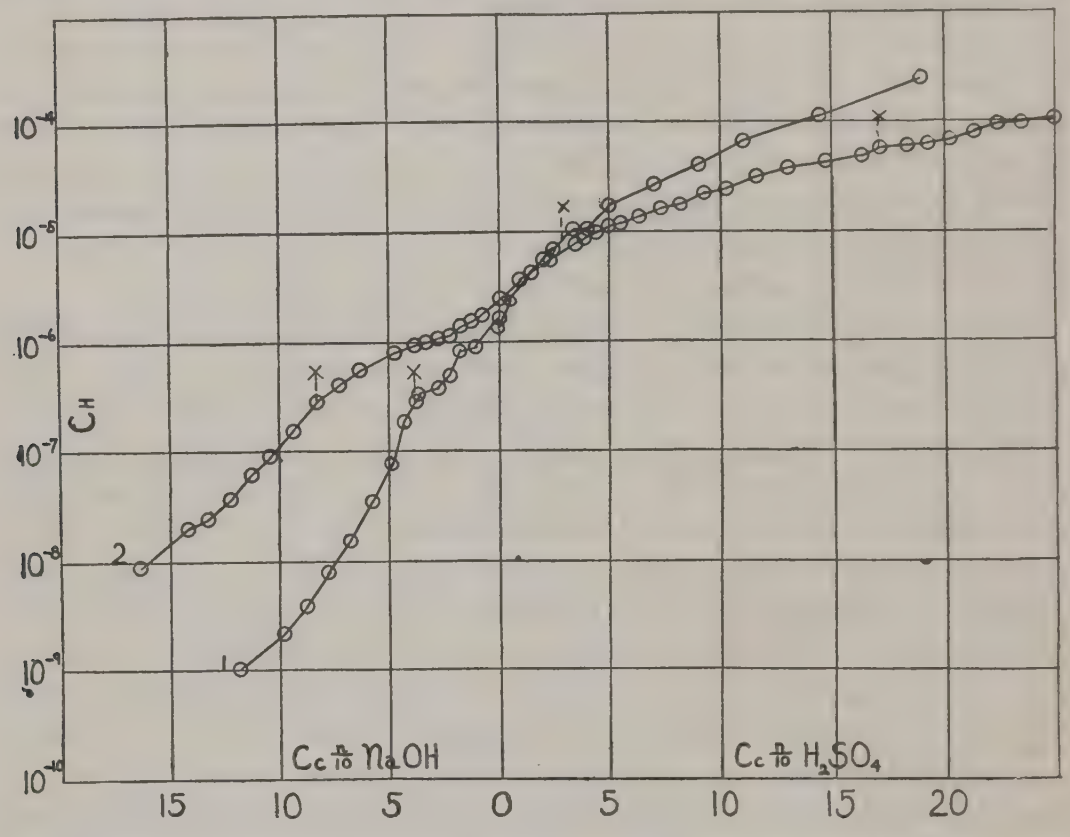

FIG. 3.—Titration graphs for cabbage juices: 1, juice from midrib: 2, juice from rest of leaf. Precipitation was observed at points marked "x." For explanation see text.

fresh petiole juice required 0.67 cc. of $N/1$ ortho-phosphoric acid (H_3PO_4) to change its acidity from 1.56×10^{-6} to 14.6×10^{-6} H^+, while the same amount of juice from the rest of the leaf required 1.48 cc. of $N/1$ ortho-phosphoric acid to change it from 2.1×10^{-6} to 14.6×10^{-6} H^+.

HYDROGEN-ION CONCENTRATION NECESSARY FOR NORMAL CONDITION OF THE PROTEINS

From figure 3 it is seen that there is an optimum hydrogen-ion concentration at which the proteins are held in solution in the cell sap. This extends from C_H $10^{-6.57}$ to about $10^{-4.3}$ when instantaneous precipitation of the proteins is taken as the measure. If a longer time is

allowed, lesser concentrations of acid precipitate the proteins. In fact, if phosphoric acid is added until the hydrogen-ion concentration is increased to the point to which it is increased by freezing, a precipitation of the protein occurs in about the time necessary to kill plants by freezing. However, changes in state much less than precipitation of the proteins may be sufficient to cause changes in permeability or even the death of protoplast.

DIFFERENCES BETWEEN THE MIDRIB AND THE REST OF THE LEAF

The foregoing data indicate that there is a difference between juices expressed from the midrib and from the rest of the leaf. The proteins in the midrib juice seem to be more easily precipitated by increase of acidity, and the juice has a lesser buffer effect. Simultaneous with the precipitation of proteins there is a decrease in acidity of the rediluted petiole juice to a value less than the original. The change of acidity is comparable to the conditions causing a change from red to blue in coleus leaves when they are thawed out. In juice from the rest of the cabbage leaf this does not occur to so great a degree. The irreversible precipitation of proteins is held to agree with the greater injury or stimulation of the cells of the midrib and those at the hydathodes than to those in the spaces between the veins, as shown by tumor formation both on freezing and on subjecting to ammonia fumes. It remains to be seen whether the cells of the midrib in plants generally may not have greater powers of regeneration than other cells; yet it is indicated by the more frequent regeneration from such cells in preference to other areas. It is suggested also that the point of regeneration may be determined by conditions similar to the above on stimulation in a similar manner by acidity increased through desiccation.

PRECIPITATION OF THE PROTEINS OF HARDENED AND NONHARDENED CABBAGE ON FREEZING

The precipitation of proteins on freezing was found by Schaffnit (*44*) to be greater in the case of juices expressed from greenhouse plants than from plants taken from the open in winter. Chandler (*5*) doubts that protein precipitation can be the cause of frost injury. However, his results show that protein precipitation occurs on freezing and in the case of hardened succulent plants to a lesser degree than in nonhardened plants. The differences in the precipitation of proteins given in his last report (*5, p. 186*) are small, but he suggests that they are within the range of experimental error. He does not state the relative hardiness of the plants used, and it is entirely possible that they were not thoroughly hardened so as to be frozen without injury.

The writer has determined the relative protein precipitation in hardened and nonhardened cabbages. Plants from the same lot were grown

part in a warm compartment of the greenhouse, part in a cold frame at temperatures usually above 0° C., but frequently low enough to freeze the plants stiff. The hardened plants were uninjured by an hour's freezing at −3°, while those from indoors were killed. Samples of juices were collected from the leaves of both hardened and nonhardened plants, using the same method for grinding and expressing in both cases. The juices were quickly frozen in Nessler tubes in a freezing mixture at −4° and kept at that temperature for two hours. Control samples were placed at once into ice water and kept there for the same time as the frozen samples. After two hours the frozen samples were quickly thawed in slightly warm water and then cooled to 0° as soon as all the ice had melted. All the samples were then placed in tubes and centrifuged together for eight minutes at high speed. Samples were withdrawn with a pipette to avoid disturbing the precipitates. The total nitrogen was determined by the Nitrogen Laboratory of the Bureau of Chemistry. On expressing the total nitrogen as proteins, it was found that 9.4 per cent of the original quantity of protein was precipitated from the hardened plants, and 31.2 per cent from the nonhardened plants by this period of freezing.

A change in state of proteins sufficient to allow these proportions to be precipitated by centrifuging the juices certainly bears some relation to frost injury. The lesser amount of precipitation in the case of juices from hardened cabbages indicates that this may account for the lesser injury to such plants.

PRECIPITATION OF THE PROTEINS OF HARDENED AND NONHARDENED CABBAGES ON THE ADDITION OF ACID

Juices were obtained in the same manner as before from hardened and nonhardened cabbages. To 50 cc. samples a sufficient quantity of acid was added to increase the hydrogen-ion concentration by approximately the amount by which it was increased by freezing at −3° C. This quantity was found by previous experiments to be 5 cc. of *N/10* sulphuric acid for 50 cc. of juice from nonhardened plants. The juices were kept in ice water for an hour, together with controls. The samples were then treated as in the previous experiment. It was found that this quantity of acid precipitated 11 per cent of the original quantity of proteins in the juice from hardened plants and 44 per cent in the juice from nonhardened plants. This indicates that the proteins are more easily precipitated by increase of the hydrogen-ion concentration in the juices of nonhardened plants than of hardened plants. This sensitiveness of the proteins to the addition of acid is closely paralleled by their sensitiveness to freezing.

PLATE 7

A.—Injected areas of cabbage leaves photographed by transmitted light immediately after freezing.

B.—Injected areas of tomato leaves photographed by transmitted light immediately after freezing. The leaf at the left shows the mottled appearance which occurs after a few days.

A
B

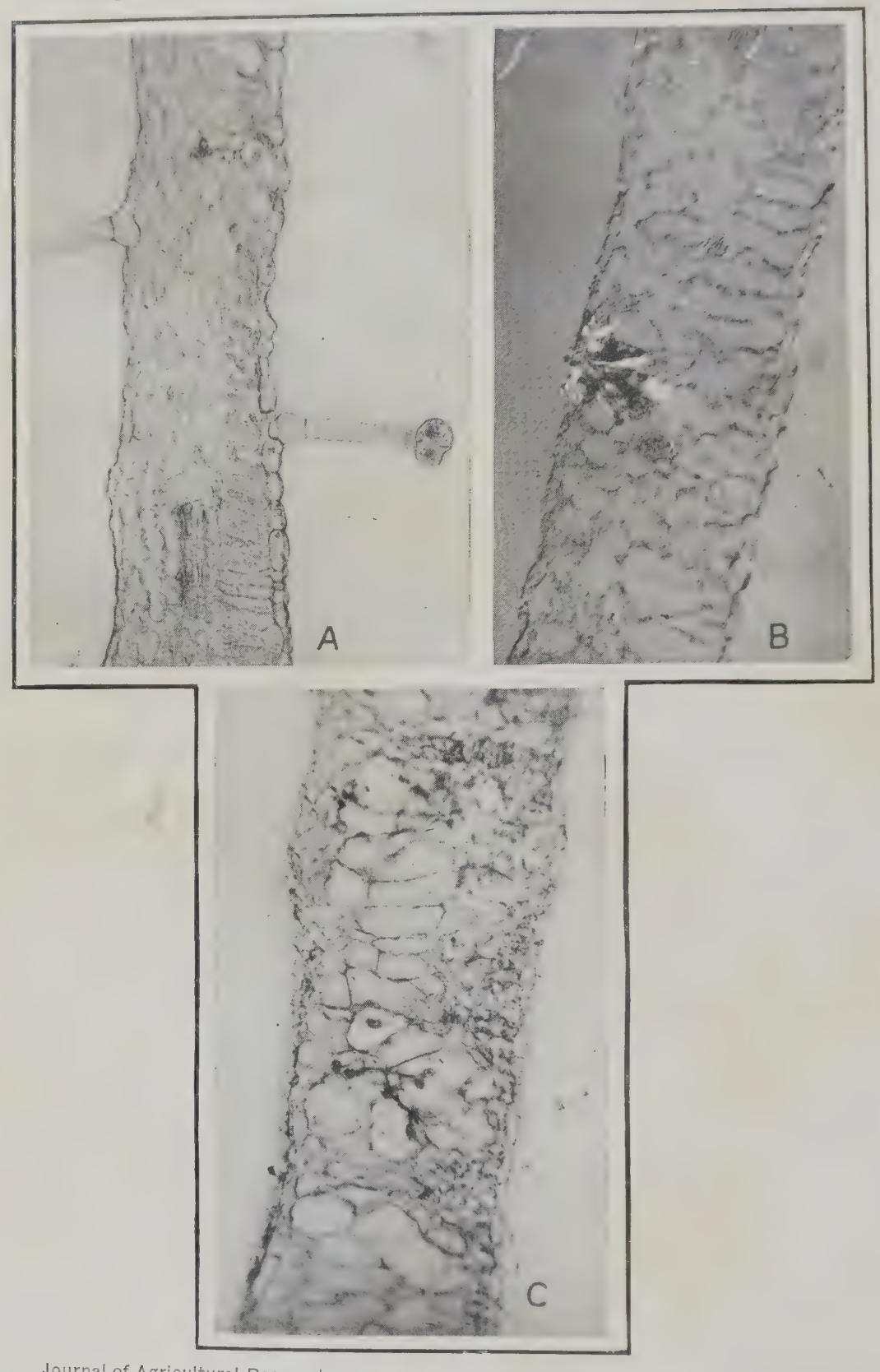
A
B
C

PLATE 8

A.—Tomato leaf showing the collapse of the palisade in the frozen areas. Note the normal condition of the cells at the right of the trichome, in which no ice formation occurred.

B.—Crystals of calcium malo-phosphate in the injected areas of cabbage leaves photographed by polarized light.

C.—Young tumor of cabbage leaf three days after freezing, showing large nuclei and multinucleate cells.

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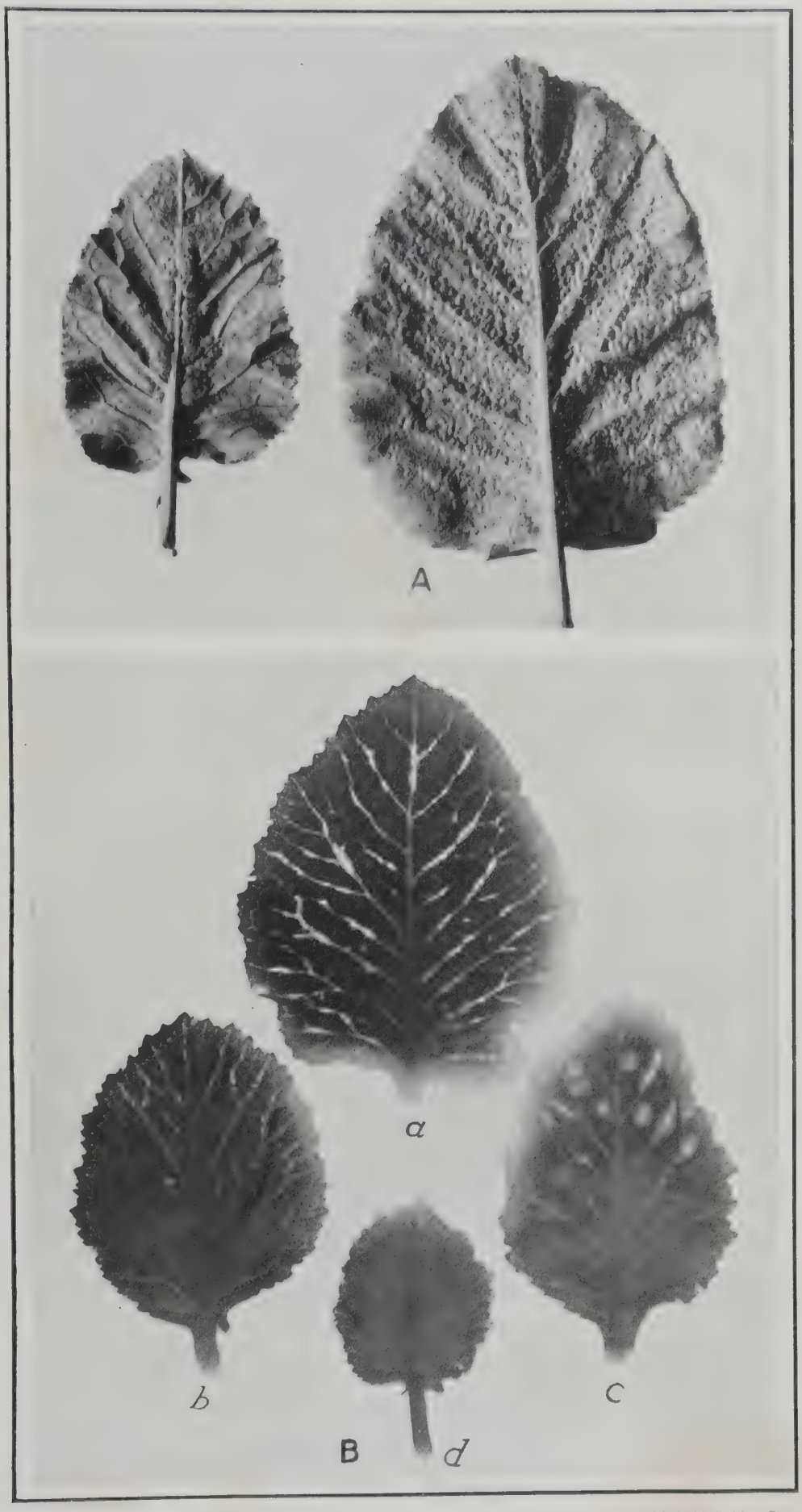
PLATE 9

A.—Distribution of the intumescences on cabbage leaves. In the leaf at the right nearly all of the leaf cells show renewed growth.

B.—Intumescences on cabbage leaves photographed by transmitted light. a, b, d show the occurrence along the veins, and c the development at the hydathodes.


A
a
b
C
B
d

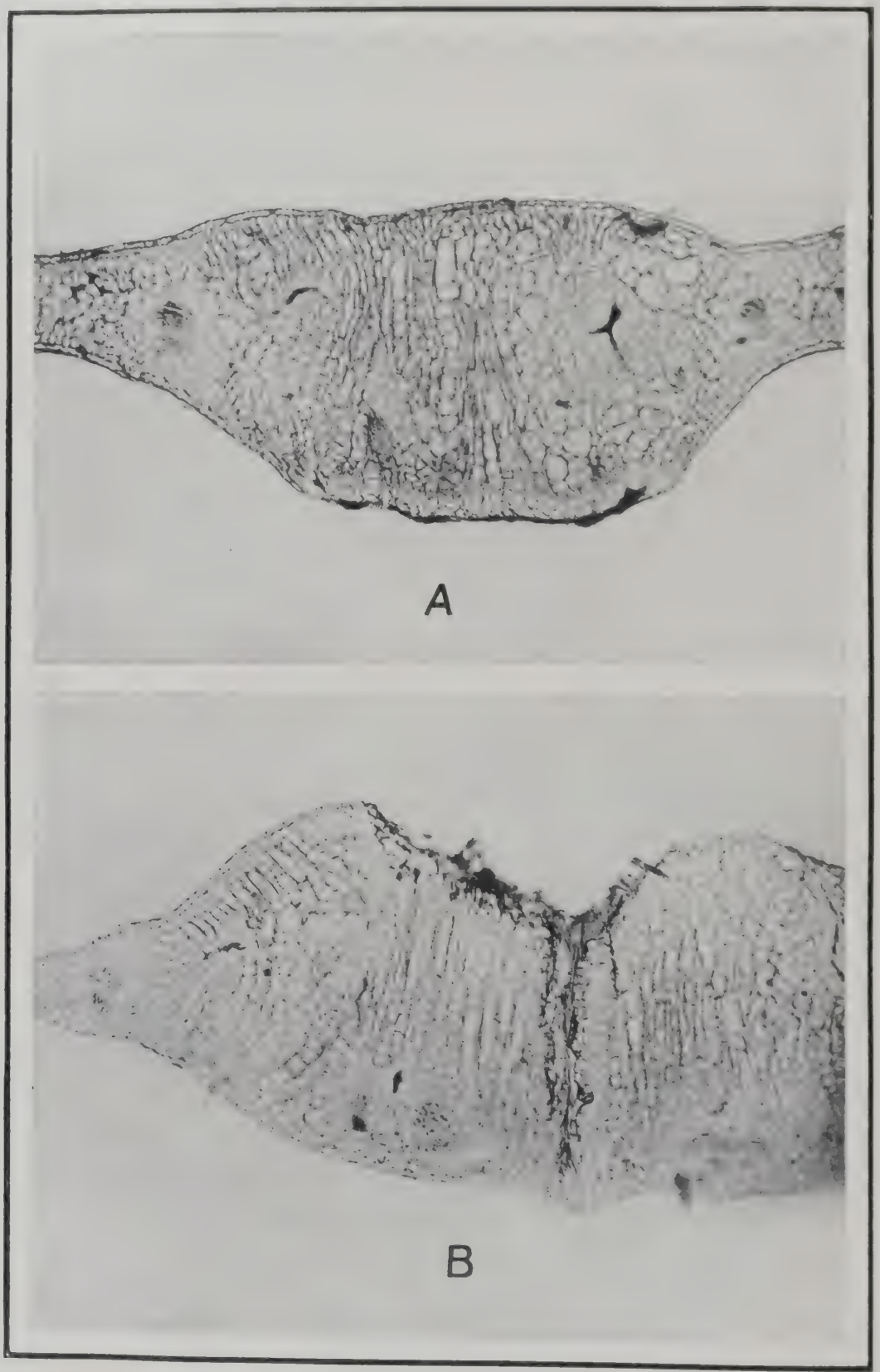

A
B

PLATE 10

A.—Section of cabbage tumor after seven days, showing the chains of cells beginning at the epidermis and areas of small cells in active division in the center of the leaf.

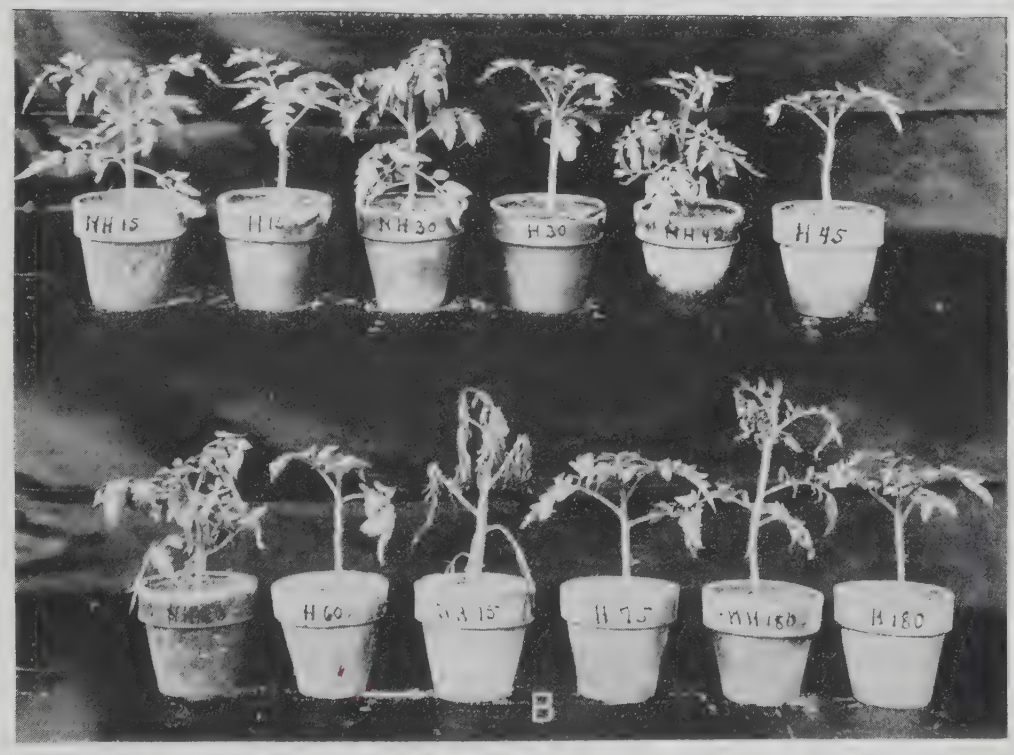
B.—Section of cabbage tumor after two weeks' growth. The epidermis has been broken at the top, and infection may occur. Some sclerenchymatous cells with thick lignified walls have developed in the center of the tumor.

PLATE 11

A.—Cabbage plants showing the relative injury to hardened (H) and nonhardened (NH) plants after exposure for the time given on the label in minutes to a temperature of $-3°$ C. Breaking of the petiole while frozen caused some of the lower leaves of the hardened plants to droop; otherwise these leaves were uninjured.

B.—Tomato plants showing the relative injury to plants from the greenhouse (NH) and from coldframes (H) after exposure for the time given on the labels in minutes to $-1.5°$ C. The plants from the coldframes were not frozen. The young leaves show the injury first.

A


NH 15
H 30
H 45
H 60
H 75
NH 180
H 180
B

DISCUSSION OF RESULTS

Haberlandt (*16*) found that a growth similar to that of the tumor cells of cabbage occurs in the storage cells which lie next to the phloem tissue in small pieces cut from potato tubers. Growth occurs in pieces 0.5 by 1 by 1 mm. only when phloem cells are present. Xylem cells are not necessary. Haberlandt stated that growth does not occur in such cells because they are physiologically different from cells farther from the phloem bundles. Neither is growth due to nutrient materials coming from the phloem; nor is it due to the phloem cells giving off substances which counteract the lethal overstimulation of the cells by wound stimulus. He attributed the growth to the excretion of a growth-producing enzym (*Wuchsenzyme*) by the phloem cells and stated that the companion cells rather than the sieve tubes are responsible for it.

In any such case or in freezing it is difficult to establish just what wound stimulus consists in. In the case examined in this investigation it appears that at least a part of the wound stimulus can be accounted for by the change of the hydrogen-ion concentration on freezing and by the consequent precipitation of proteins. This offers a tangible thing to which wound stimulus can be ascribed. It is possible also that other wound stimuli may be caused in a similar manner by desiccation. In nearly all such cases it appears that a stimulus which causes growth on moderate application will cause death on overstimulation of the cells. Thus, the first layers of a cut surface of potato die, and phellogen formation is taken up by the lower layers. Similarly, cabbage cells are either stimulated to growth or killed outright, according to the degree of freezing. The greater stimulation of the tissue about the veins to form tumors on freezing is possibly due to the fact that such cells are more easily injured than the other cells of the leaf, and thus receive a stronger stimulus. The easier precipitation of the proteins in juices expressed from the midrib than from the rest of the leaf indicates such a physiological difference.

Cases of renewed growth or regeneration, such as shown by Klebs (*19*) and Miehe (*35*) to be produced on plasmolysis, have usually been ascribed to removal of the cells affected from the influence of those about them—that is, to a removal of the correlation effect. It has been reported by Klebs (*19*) that growth after plasmolysis generally occurs only in the algæ. Miehe (*35*) states that in such plasmolyzed cells only the living continuity is destroyed. This statement is taken to refer to the plasmodesmen. It has been shown by Gardiner (*10*) and others (*11*) that there is a continuity of protoplasm between adjacent cells. Gardiner (*10*) found that these threads of protoplasm were not broken in every case during plasmolysis, but fine strands could be observed running out to the cell wall. Mangham (*25*, *26*) ascribes to the plasmodesmen an important function in conducting sugar from cell to cell. Czapek (*7*)

showed that conduction of food materials occurred after the plasmolysis of these conducting cells. The protoplasmic connection between cells is, therefore, not necessarily broken by plasmolysis, according to the evidence above cited. Consequently it is necessary to assume that in some manner these strands are killed on plasmolysis if the growth stimulus is attributed to the removal of correlation factors and the breaking of protoplasmic connection between the cells.

It is entirely possible that the strands running to the cell wall in plasmolysed cells may remain alive, since they are scarcely thinner than the plasmodesmen themselves. In this case the "living continuity" is not broken. It is equally possible that they are killed, and in such case death may be due to changes in the state of the protoplasm constituents owing to changed acidity and an increase in the concentration of the salts. If these threads are killed on plasmolysis of the cells, equal changes can be expected to occur on the surface of the protoplast. It was Chandler's idea that—

> killing from cold is more likely a mechanical injury due to the withdrawal of water from the protoplasmic membrane than an injury resulting from a precipitation of proteins.

The effect of plasmolysis on the regeneration of plasmolyzed cells can then be ascribed also to this change in the membrane as well as to the removal of the correlation effect. If growth is due to the removal of the correlation effect through plasmolysis of the cells on freezing then one would expect the plasmolyzed cells of the hardened cabbages to be stimulated to growth in the same manner as those of nonhardened cabbages. But this does not occur; consequently the renewal of growth in the cells can not be ascribed to the breaking of the living continuity and removal of the correlation effect in the case of the cabbage. The lack of growth in the cells of hardened cabbages is evidently due to their inability to withstand the concentration of the cell sap and increased acidity without injury.

It appears that in the process of hardening there are changes which occur in the proteins. To these changes the greatest effect of the hardening is to be ascribed. The changes are cleavages to simple forms if the increase of amino acids on hardening of the plants is taken as an indication. The simple forms of the proteins are not so easily precipitated by freezing or by the addition of acid as the more complex forms. The increase of acidity of a plant juice on freezing is accompanied by an increase in the concentration of the salts. Both conditions are favorable for protein precipitation.

The surface layer is the most exposed part of the plasmolyzed protoplast. It is therefore probable that the greatest changes in aggregation of the proteins occur there.

The change in color of coleus leaves after freezing indicates that through irreversible combination with the proteins the excess hydro-

gen-ion concentration is decreased on thawing. This is probably the case in the frozen cells of the injected areas of nonhardened cabbages. In those cells which survive one would then expect to find a slightly decreased acidity. No indicator covering the proper range of acidity has been found which will penetrate the cabbage cells in sufficient concentration to show a color deep enough not to be masked by the chlorophyll. However, such a change is indicated by the change of the injected spots on coleus leaves from red to blue. The effect of the decreased acidity may be to cause greater activity of the peroxidase present or to allow its accumulation. Blackening occurs in the areas of *Aucuba japonica* injected by freezing, indicating an increased activity of the oxidizing enzyms. Krasnosselsky (*22*) reports an increase in the concentration of the oxidizing enzyms due to wound stimulus. It is known that the oxidizing enzymes are destroyed quite rapidly at the hydrogen-ion concentration shown by cabbage leaves and that a decrease in acidity favors their action.

SUMMARY

(1) The first indications of frost injury to succulent plants were observed in the appearance of injected areas over the leaf surface. These injected areas are caused by the withdrawal of water from the protoplast and the displacement of air in the intercellular spaces. Inoculation of the undercooled leaf tissue from ice formed on the surface is generally the cause of the local freezing. Wax on the leaf surface prevents the inoculation of the undercooled tissue and thus prevents injury from freezing.

(2) Frozen cells in the leaves of cabbage, bryophyllum, salvia, and lettuce were found to be stimulated to growth and to produce tumors similar to those shown in pathological conditions, but without the presence of bacteria. Frozen spots on the leaves of tomato, coleus, geranium, and a number of other plants did not receive a growth stimulus as in the former cases, but were killed by the freezing. This local killing of the tissue gives such leaves a spotted appearance.

(3) The peroxidase content of the intumescences of cabbage induced by freezing was found to be much greater than that for normal leaf tissue. A decrease in the hydrogen-ion concentration may occur in such cells, and this condition may allow greater activity or accumulation of the respiratory enzyms, especially peroxidase.

(4) It is suggested that the growth stimulus in frozen cells is not due to the removal of the correlation effect on plasmolysis of the cells, but is to be ascribed to a partial precipitation of the proteins of these cells. This precipitation results in an increase in the permeability of the cells to water, and in the ability of the cells to hold sugars.

(5) The apparent osmotic pressure on plasmolysis of the tumor cells was found to be less than that for normal mesophyll cells. However, the tumored areas of the leaf do not freeze more readily than the other areas.

(6) The principal effect of the hardening process for cabbages is a change in the constituents of the protoplasm which prevent their precipitation as a result of the physical changes incident upon freezing. The proteins are changed to forms which are less easily precipitated. This is indicated by an increase in the amino-acid content of the cabbage plants on hardening.

(7) The factors which produce protein precipitation on the freezing of a plant juice are held to be principally the increase in the hydrogen-ion concentration and the increase in the concentration of the salts. The latter factor is held to be insufficient to cause precipitation except under the conditions of a changed acidity. Cabbage plants were found to become resistant to a half-hour's freezing at $-3°$ C. after exposure to $+3°$ for five days. During this time the carbohydrate changes were slight. Hence, the prevention of protein precipitation by sugar accumulated during hardening is not sufficient to account for the resistance of hardened plants to freezing.

(8) The proteins of the midrib of cabbage leaves are precipitated more readily than those from the rest of the leaf. This is considered to be due to physiological differences between vascular tissues and the other tissues of the leaf.

(9) In juices of nonhardened and hardened cabbages the proteins of the former were found to be precipitated to a greater degree by freezing than those of the latter. The percentage of precipitation for such juices on freezing is closely paralleled by the relative precipitation on the addition of acid.

(10) The greatest changes induced by freezing are supposed to occur n the outer portions of the protoplast since this is most exposed on plasmolysis.

(11) The effects of desiccation, freezing, and plasmolysis are considered to be similar, in that all these processes cause changes in the hydrogen-ion and salt concentrations.

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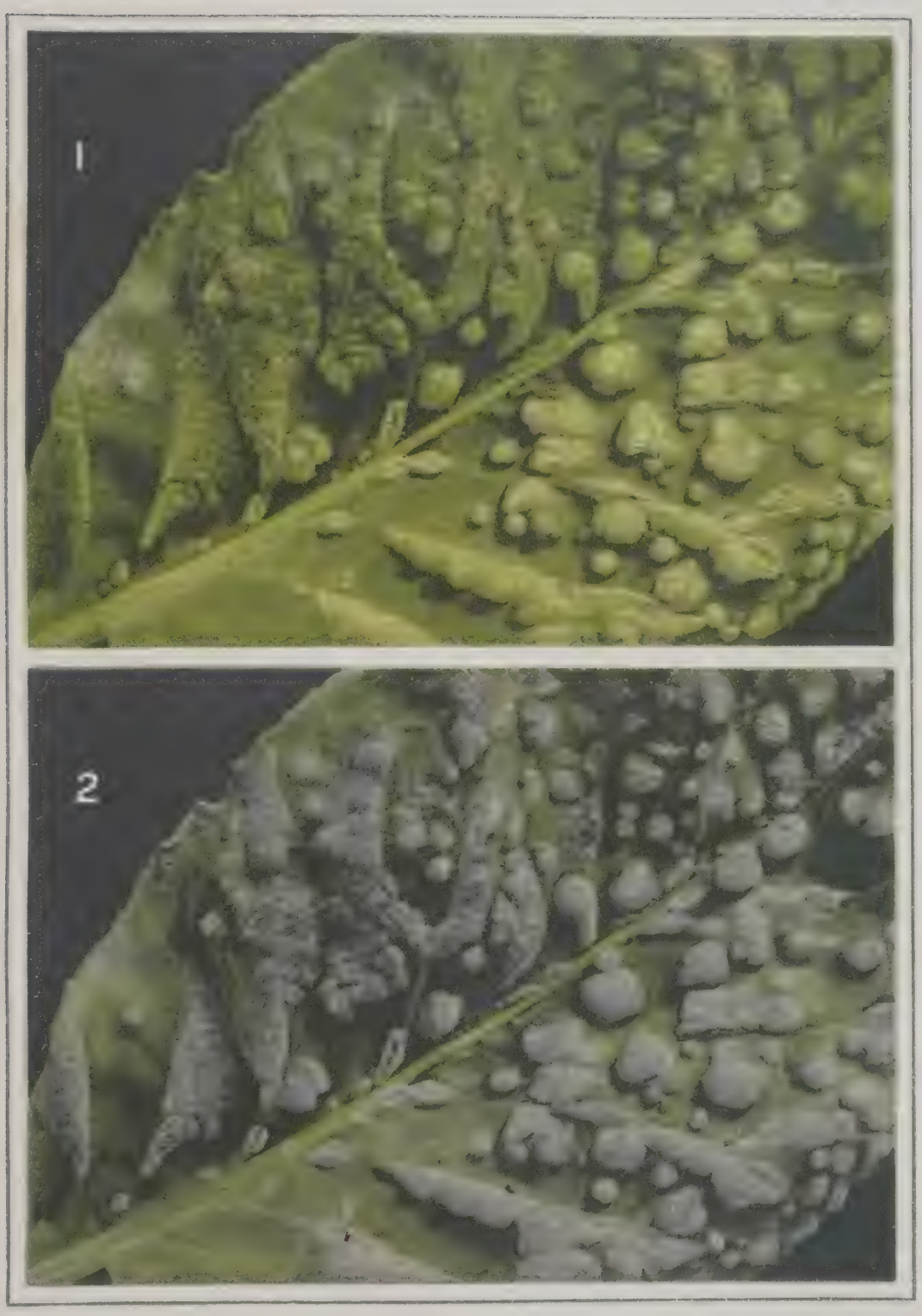
PLATE A

1.—A cabbage leaf showing the chlorophyll distribution in the intumescences.

2.—A cabbage leaf showing the comparative peroxidase reaction given in the tumor and leaf cells with tetramethylparaphenylenediamine.


1
2

CHEMISTRY OF SWEET-CLOVER SILAGE IN COMPARISON WITH ALFALFA SILAGE

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INTRODUCTION

In pursuing chemical studies in making alfalfa silage, previously reported,[1] there were certain phases which seemed to warrant further investigation. In outlining the plan for this additional work it seemed desirable to broaden the investigation by including sweet clover. As in the previous investigations, milk bottles were used as containers.

PLAN OF THE WORK

After the alfalfa (*Medicago sativa*) or the sweet clover (*Melilotus* spp.) had been cut, it was allowed to wilt in the sun for about two hours. The alfalfa was cut in one-tenth bloom, the sweet clover just before bloom. The alfalfa was of average size, fine quality, first cutting. The sweet clover was very rank and had a high moisture content. After wilting, the material was brought to the laboratory and passed through a small feed cutter. Alfalfa was used alone. The sweet clover was used alone and also with ground corn in the proportion of 1 to 10. Each bottle was weighed before filling. All were packed full, closed with corks which were wired, and sealed with wax such as is used in closing desiccators.

Three bottles, one of each kind, were opened each day for the first week, then every other day for the second week, then each week for the next four weeks, and finally each month as long as needed. On opening, the silage was judged as to color and grade. All the bottles, without exception, had first-class silage. In this study an effort was made to obtain as nearly as possible the same grade of silage in all the bottles. From our previous work the conditions for obtaining this result were known.

LOSS OF MATERIAL

As soon as a bottle was opened the content was weighed. The weight of the material put into the bottle, about 700 gm., had been obtained at the time of filling. From these figures were calculated the loss of material during silage making. The figures obtained are given in Table I.

[1] SWANSON, C. O., and TAGUE, E. L. CHEMICAL STUDIES IN MAKING ALFALFA SILAGE. *In* Jour. Agr. Research, v. 10, no. 6, p. 275–292. 1917.

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TABLE I.—*Loss of weight, in grams, of various kinds of silage*

Age of silage.	Alfalfa alone.	Sweet clover alone.	Sweet clover and corn-chop.	Age of silage.	Alfalfa alone.	Sweet clover alone.	Sweet clover and corn-chop.
Days.				*Days.*			
1	2.0	6.0	1.0	13	4.0	4.0	6.0
2	5.8	6.0	.....	15	6.0	5.0	9.0
3	6.8	6.5	6.0	17	9.8	6.6	10.5
4	5.0	8.0	7.3	21	7.0	8.1	39.0
6	8.0	7.0	6.0	29	7.1	7.6	9.8
7	9.0	.....	.....	43	8.3	23.3	1.6
8	8.0	6.0	.....	64	7.7	10.5	9.0
10	6.0	7.0	5.8	98	7.0	8.6	7.0

These results show that the losses, with a few exceptions, were approximately 1 per cent. The greater part of the larger losses was silage juice. The gas generated in the bottle would push out the stopper slightly, resulting in loss. Since the total loss is so small, the losses due to fermentation are insignificant.

MOISTURE CONTENT

The moisture content in the silage was determined on 100-gm. samples, using the material in the condition in which it was taken from the bottles. The moisture percentages are given in Table II.

TABLE II.—*Percentage of moisture in alfalfa silage*

Age of silage.	Alfalfa alone.	Sweet clover.	Sweet clover and corn-chop.	Age of silage.	Alfalfa clover.	Sweet clover.	Sweet clover and corn-chop.
Days.				*Days—Contd.*			
0	63.2	77.5	71.2	15	64.5	77.0	71.4
1	63.1	77.3	71.2	17	64.1	75.9	72.2
2	64.3	75.4	71.4	21	63.6	77.0	72.7
3	62.2	75.9	71.9	29	62.1	77.1	72.5
4	63.7	77.3	73.0	43	62.1	76.1	72.6
6	64.6	76.7	71.2	64	62.4	77.4	72.9
7	64.8	.....	.....	98	62.9	77.5	73.2
8	64.6	76.5	73.3				
10	64.9	77.4	72.1	Average	63.6	76.5	72.1
13	64.9	77.8	71.7				

The general agreement of these figures for each kind of silage shows that the material in the bottles was fairly uniform.

METHOD OF MAKING THE WATER EXTRACT OF SILAGE

When each bottle was opened, 100 gms. of silage were weighed into a quart Mason jar and 430 cc. of carbon-dioxid-free water added, so as to make the total moisture content very nearly 500 cc. Thus, each 5 cc.

represented the extract from 1 gm. of silage. This was on the assumption that the average moisture content was 70 per cent. This method of procedure involved a slight error which is without significance in this work. The jar was closed and placed on a shaking machine for two hours, after which the material was strained through a linen cloth into a 500-cc. centrifuge cup, and centrifuged for five minutes at the rate of 2,400 revolutions per minute. The average relative force, times gravity, is 1,344 at this speed, according to the statement of the manufacturer. This gave a clear but dark-colored supernatant liquid. In order not to stir up the sediment in the bottom of the cup, it was necessary to allow the centrifuge to come to a stop without using the brake. Because of the presence of some light particles which would not settle to the bottom, it was necessary to filter on folded filters. This filtrate was then used for the following determinations:

(1) Acidity by titrating to phenolphthalein.

(2) Acidity by using the hydrogen electrode.

(3) The nitrogen in amino form, by titrating in the presence of formaldehyde using thymolphthalein as indicator.

(4) The nitrogen in amino form by titrating in the presence of formaldehyde to a certain hydrogen-ion concentration, using the hydrogen electrode.

(5) Total nitrogen in the water extract.

(6) Total nitrogen in water extract not precipitated by phosphotungstic acid.

An attempt was made to determine nitrogen in the water extract by Stutzer's method, but the amounts were so small that differences were within the analytical error.

METHOD OF MAKING THE ALCOHOLIC EXTRACT OF SILAGE

When each bottle was opened, 100 gms. of the silage were weighed into a quart Mason jar and 250 cc. of 95 per cent alcohol added. The jar was then sealed and allowed to stand till a convenient time for doing the work. This was necessary, as the work on the water extract had to be finished at once, and occupied the available time. On the basis of total moisture in the silage, previously determined, enough carbon-dioxid-free water was added to the jar to make the volume 500 cc. This would make the percentage of the alcohol about 50. After adding this water the jar was sealed, shaken, and allowed to stand for an hour, when the material was strained through linen cloth. From this point the precedure was the same as with the water extract, and the same chemical determinations were made.

ACIDITY IN THE WATER AND THE ALCOHOLIC EXTRACTS OF SILAGE, TITRATING TO PHENOLPHTHALEIN

Twenty-five cc. of the water or the alcoholic extract, representing 5 gms. of silage were pipetted into a 500-cc. Jena Erlenmeyer flask, and 200 cc. of carbon-dioxid-free water were added. One cc. of phenolphthalein as indicator was used. The extract was then titrated to a faint pink with *N/20* sodium hydroxid. The results obtained are presented in Table III.

TABLE III.—*Acidity in water and alcoholic extracts of silage*

[Results expressed as cubic centimeters of *N*/20 sodium hydroxid on the extract from 5 gm. of silage titration with phenolphthalein as indicator]

Age of silage.	Alfalfa alone.		Sweet clover alone.		Sweet clover and corn-chop.	
	Water.	Alcohol.	Water.	Alcohol.	Water.	Alcohol.
Days.						
0	3.3	7.7	2.8	3.6	3.3	3.8
1	9.0	11.7	4.6	6.1	5.3	5.7
2	10.4	12.0	3.4	7.2	5.3	6.9
3	11.4	12.9	6.3	8.7	5.6	7.6
4	9.1	13.5	8.7	10.1	8.2	10.0
6	10.6	14.9	8.0	9.4	9.0	10.9
7	14.4	15.4	....	....	....	....
8	11.5	15.0	6.4	9 9	13.2	14.8
10	13.4	16.9	11.6	12.5	9.3	13.2
13	17.8	19.0	11.3	12.8	13.2	16.1
15	18.5	19.9	10.3	13.6	14.3	17.3
17	16.6	20.8	12.2	14.7	14.8	17.3
21	18.1	22.4	13.8	15.5	16.6	20.0
29	20.8	25.2	13.0	16.5	17.0	21.0
43	26.6	28.4	11.7	16.5	16.8	22.4
64	25.7	30.4	12.6	17.7	18.9	25.2
98	26.7	....	12.8	18.5	18.6	26.3

The figures in Table III show the following results:

(1) The acidity in the alcoholic extract appears to be uniformly greater than in the water extract. The silage made from sweet clover alone and from sweet clover plus corn meal show the same relative differences in the acidity of the alcoholic and water extracts as the silage made from alfalfa alone. That the alcoholic extract from corn silage will give a larger percentage of acidity than will the water extract when phenolphthalein is used as the indicator has been shown in a previous publication from this laboratory.[1] A suggestion was reported in that journal that the greater acidity of the alcoholic extract is due to fatty acids liberated by lipases which are active in silage formation. That this explanation is probably not correct will be shown in a subsequent part of this paper.

[1] SWANSON, C. O., CALVIN, J. W., and HUNGERFORD, Edwin. ACIDITY IN SILAGE: METHOD OF DETERMINATION. Jour. Amer. Chem. Soc., v. 35, no. 4, p. 476–483. 1913.

(2) The acidity of matured silage made from alfalfa alone is twice that of matured silage made from sweet clover alone, as determined in the water extracts. As determined in the alcoholic extract, the acidity is approximately one-third greater.

(3) The comparatively smaller acidity of the silage made from sweet clover may be partly due to the high moisture content. In a previous investigation[1] it was found that silage made from freshly cut alfalfa had a lower percentage of acidity than that made from wilted alfalfa. While the sweet clover used in the present investigation was wilted, it still had a large moisture content.

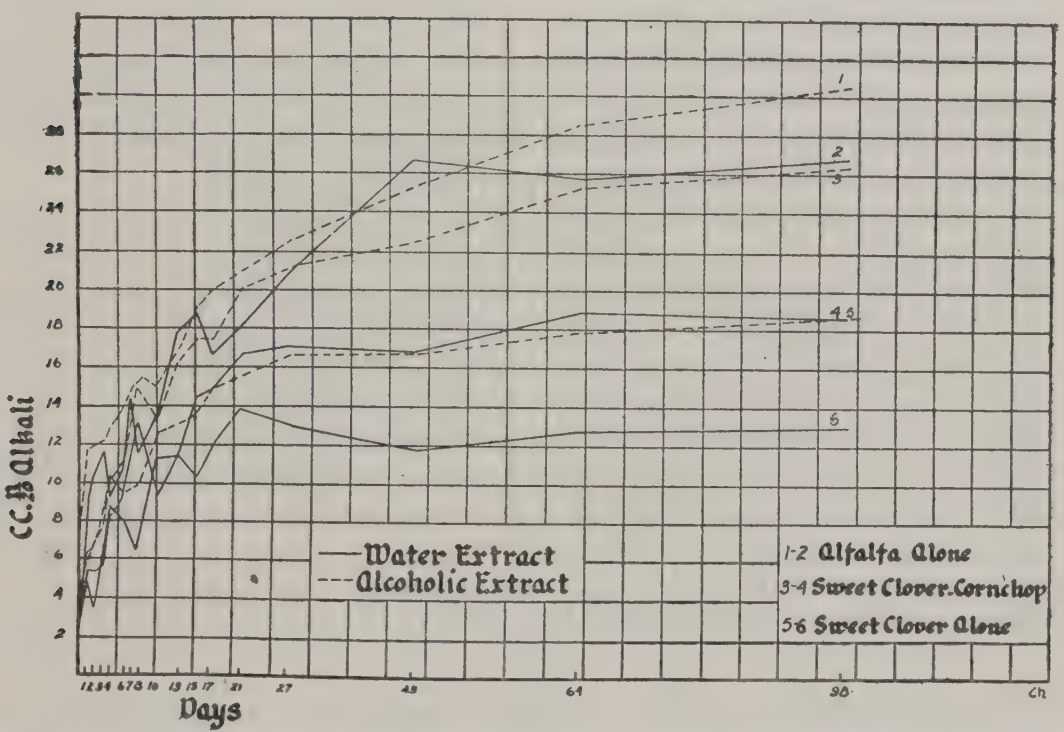

FIG. 1.—Graphs showing the acidity in water and alcoholic extracts of silage; obtained by colorimetric titration with phenolphthalein.

(4) The acidity of the water-extract silage from alfalfa alone is the same as the acidity of the alcoholic extract of silage from sweet clover plus corn meal.

(5) When corn meal was added to sweet clover, the acidity was increased about 50 per cent. This is in harmony with results obtained in a previous investigation[2] with adding corn meal to alfalfa in making silage.

(6) About two-thirds of the total acidity is developed in the first 15 days. The maximum acidity is reached in about 40 to 60 days. (See fig. 1.)

[1] SWANSON, C. O. and TAGUE, E. L. OP. CIT., p. 281.
[2] SWANSON, C. O., and TAGUE, E. L. OP. CIT., p. 282.

TITRATION TO A CERTAIN HYDROGEN-ION CONCENTRATION WITH THE HYDROGEN-ELECTRODE APPARATUS

The general arrangement of the apparatus for determining the hydrogen-ion concentration by the use of the hydrogen electrode was that given by Hildebrand.[1]

Our outfit contains the following pieces: One Weston D. C. milli-voltmeter and multiplier; one Kohlrausch slide wire bridge with extension coils; one Leeds and Northrup No. 2,500 type R. galvanometer with lamp and scale; two Edison storage batteries. The calomel and hydrogen electrodes were made according to the directions given by Hildebrand.

For the first part of this work neither this bridge nor the galvanometer were available. The bridge used was a straight-wire bridge such as is commonly found in physics laboratories. Instead of the galvanometer we used the Lipmann electrometer and reading telescope.

The galvanometer with the lamp and scale, as well as the Kohlrausch bridge, are found exceptionally satisfactory for this class of work.

As a source of hydrogen we have found that made by the electrolytic process very satisfactory. This can be bought compressed in iron cylinders. As a precaution against impurities the gas is washed in a train of alkaline pyrogallic acid, potassium permanganate, mercuric chlorid, and carbon-dioxid-free water. The hydrogen made from aluminium and sodium hydroxid was found just as satisfactory, but more expensive as to materials and labor.

METHOD OF DETERMINING THE TITRABLE ACIDITY BY THE USE OF THE HYDROGEN ELECTRODE

Twenty-five cc. of the water or alcoholic extract were pipetted into a wide mouth 250-cc. flask. The calomel and the hydrogen electrodes were inserted through a rubber stopper which fitted the mouth of the flask. As the lower end of the hydrogen electrode was bell-shaped, it was necessary to use a partially split stopper. Through a third hole in the stopper the tip of a burette was inserted so that the N/20 sodium hydroxid could be added without exposing the contents of the flask to the air. Hydrogen gas was then allowed to bubble through the solution with frequent shaking and the bridge adjusted until equilibrium was obtained—that is, until no current flowed through the galvanometer. At this point the reading of the milli-voltmeter was noted. Next, *N/20* sodium hydroxid was added from the burette slowly with frequent shaking. It was desired to measure the amount of alkali necessary to add in order to obtain three different hydrogen-ion concentrations: $P_H 7$,

[1] HILDEBRAND, J. H. SOME APPLICATIONS OF THE HYDROGEN ELECTRODE IN ANALYSIS, RESEARCH AND TEACHING. *In* Jour. Amer. Chem. Soc., v. 35, no. 7, p. 847–871, 15 fig. 1913.

the true neutral point; $P_H 8.3$, the point of color change for phenolphthalein; and $P_H 9.3$, the point of color change for thymolphthalein. The *N/20* sodium hydroxid was added until equilibrium was reached for each point and the total cc's noted. When the last point was reached, 25 cc. of formaldehyde solution which had previously been neutralized to a hydrogen-ion concentration of $P_H 9.3$ was added and *N/20* sodium hydroxid added again until the potential indicated a concentration of $P_H 9.3$. This last operation gave the figures for calculating the titrable nitrogen.

The number of cubic centimeters of *N/20* sodium hydroxid necessary to titrate to the true neutral point, $P_H 7$, also to the color change of phenolphthalein $P_H 8.3$, and to the color change of thymolphthalein, P_H 9.3 are given in Table IV.

TABLE IV.—*Quantity, in cubic centimeters, of N/20 sodium hydroxid necessary to titrate to three different hydrogen-ion concentrations, P_H 7 P_H 8.3, and P_H 9.3*

[Each sample represents the extract from 5 gm. of silage]

TITRATION TO $P_H 7$

Age of silage.	Alfalfa alone.		Sweet clover alone.		Sweet clover and corn-chop.	
	Water.	Alcohol.	Water.	Alcohol.	Water.	Alcohol.
Days.						
0	2.3	4.2	1.0	1.9	1.4	2.2
1	6.0	5.3	2.7	2.6	2.9	2.5
2	6.3	7.5	3.6	3.9	3.5	3.6
3	6.1	7.8	4.6	5.2	4.3	4.1
4	4.8	7.9	6.9	6.0	8.0	6.0
6	5.7	7.5	10.3	5.4	8.0	6.2
7	9.6	7.6	.....	.....	.....	.....
8	8.1	9.6	7.0	5.7	10.2	9.6
10	9.4	10.4	10.2	8.3	9.6	8.4
13	10.0	11.1	9.8	8.6	12.0	11.1
15	12.0	12.0	8.6	9.0	12.1	12.5
17	11.6	13.0	10.6	10.6	11.7	13.0
21	13.1	14.2	11.8	10.8	15.2	14.9
29	16.0	17.0	11.5	11.2	15.0	15.1
43	19.0	21.3	10.0	11.2	14.7	16.7
64	19.4	22.6	10.5	11.7	16.5	19.0
98	22.0	24.7	10.1	13.0	17.3	20.1

TABLE IV.—*Quantity, in cubic centimeters, of N/20 sodium hydroxid necessary to titrate to three different hydrogen-ion concentrations, $P_H 7$ $P_H 8.3$, and $P_H 9.3$*—Continued

TITRATION TO $P_H 8.3$

Age of silage.	Alfalfa alone.		Sweet clover alone.		Sweet clover and corn-chop.	
	Water.	Alcohol.	Water.	Alcohol.	Water.	Alcohol.
Days.						
0	3.3	6.3	2.9	3.2	3.1	3.3
1	8.6	8.5	5.1	4.3	6.2	4.9
2	11.0	11.0	6.7	6.1	6.9	5.4
3	11.1	11.1	8.1	7.4	8.0	6.5
4	8.4	11.3	10.4	8.4	11.5	8.2
6	10.1	11.3	13.0	7.7	11.0	8.8
7	14.2	11.2	.....	.....	.....	.....
8	11.8	13.2	9.3	8.0	13.0	12.4
10	13.5	14.0	12.5	11.1	12.6	11.1
13	13.5	14.8	12.2	11.4	14.6	14.2
15	15.0	15.8	11.5	11.7	14.8	15.1
17	14.7	16.8	13.3	13.6	14.4	15.6
21	21.6	18.1	15.1	14.0	19.8	18.0
29	21.8	21.5	14.5	14.3	18.5	18.2
43	26.0	25.7	13.8	14.1	18.7	20.1
64	26.9	26.4	14.0	14.7	21.4	23.0
98	26.5	29.2	12.7	15.9	21.8	23.7

TITRATION TO $P_H 9.3$

0	5.1	9.4	6.3	5.2	5.0	5.2
1	12.2	14.3	11.3	7.9	10.9	8.5
2	18.8	17.3	12.2	10.5	12.2	9.9
3	19.0	18.0	13.5	12.1	13.1	11.7
4	15.0	18.5	17.4	14.4	15.2	13.8
6	16.8	19.2	17.8	12.8	16.4	15.0
7	19.6	19.0	.....	.....	.....	.....
8	17.7	23.5	14.3	13.7	18.7	18.2
10	22.4	23.2	17.7	17.4	18.0	17.7
13	21.3	24.3	17.7	17.4	22.3	21.4
15	23.7	25.0	19.3	18.4	24.6	21.5
17	27.5	27.1	20.1	20.8	24.0	23.2
21	31.8	28.2	22.6	21.4	28.7	25.8
29	33.5	33.4	24.1	22.6	26.0	26.7
43	36.1	38.0	20.8	22.2	26.0	28.0
64	37.9	38.8	21.1	23.5	28.5	31.8
98	39.1	42.5	19.5	24.4	26.9	32.7

The data presented in Table IV show the following results:

(1) The acidity values obtained by the electrometric titration were practically the same for the alcoholic extract as for the water extract. In this respect it differs fundamentally from the colorimetric titration.

(2) Titrating to the true neutral point, $P_H 7$, gave a much lower acidity value than the colorimetric titration (fig. 2). Compare Tables III and IV, part 1.)

(3) Titrating to the hydrogen-ion concentration, $P_H 8.3$, or the point of color change for phenolphthalein, gave practically the same acidity value as was obtained in the water extract with colorimetric titration. (Compare Tables III and IV, part 2.)

(4) Titrating to the hydrogen-ion concentration $P_H 9.3$, or the point of color change for thymolphthalein, gave an acidity value about twice that obtained by titrating to the strictly neutral point, $P_H 7$.

(5) The acidity value of both the water and the alcoholic extracts obtained by titrating to the hydrogen-ion concentration $P_H 8.3$ agreed substantially with the value obtained in the water extract by the colorimetric titration. (See Table III.)

(6) The hydrogen-ion concentration of the water extract and the alcoholic extract agree substantially for the three points determined.

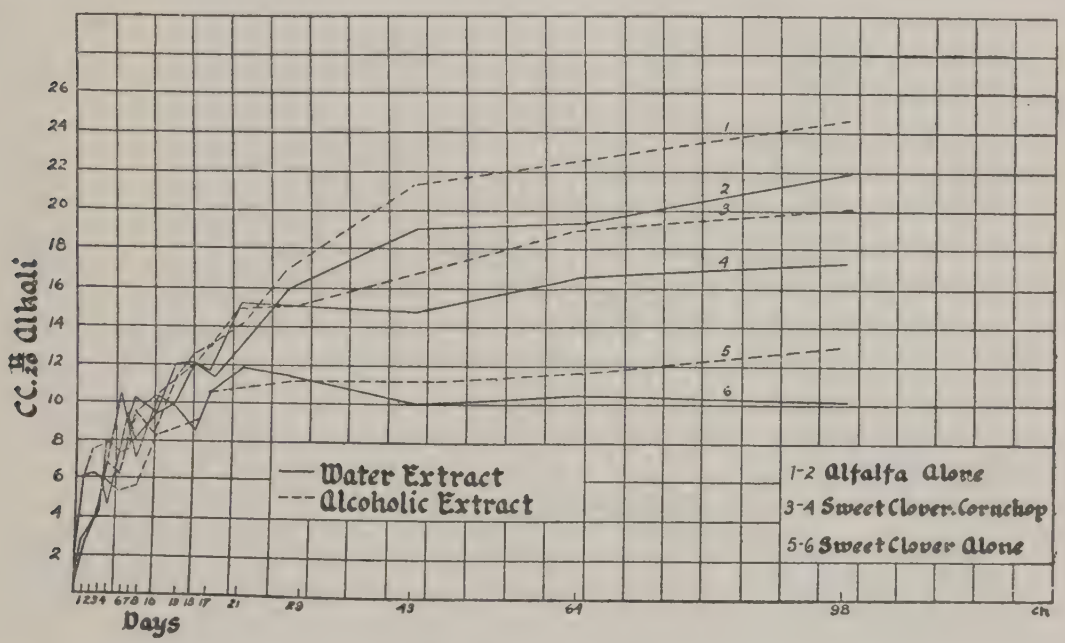

FIG. 2.—Graphs showing the quantity, in cubic centimeters, of alkali used in water and alcoholic extracto of silage; obtained by electrometric titration to $P_H 7$.

(7) These results show that the acidity value in an alcoholic extract of silage obtained by titrating to the color change of phenolphthalein are high in comparison with the true acidity value—that is the hydrogen-ion concentration. The results obtained by the colorimetric titration on the water extract correspond more nearly to the true acidity value than those obtained on the alcoholic extract. It has previously been suggested that the higher acidity value of the alcoholic extracts are due to the presence of fatty acids which are soluble in alcohol, but not in water. If this was the case the hydrogen-ion concentration of the water extract should be less than that of the alcoholic extracts. But the data show that this is not so. The higher acidity value obtained in the alcoholic extracts when the colorimetric titration is used is probably due only to the masking of the end point. The alcoholic extract contains more of

highly colored substances in solution than the water extract. That this was the case was suspected in our former investigation. Concerning this we said [1]:

> The figures for acidity are probably large. . . . . The extract is highly colored, and the end point is not easily read.

DIFFERENCES IN TITRATING TO THE HYDROGEN-ION CONCENTRATION $P_H8.3$ IN COMPARISON WITH P_H7; ALSO $P_H9.3$ IN COMPARISON WITH $P_H8.3$

By substracting the figures in the first part of Table IV from the corresponding figures in the second part, the differences between the quantity of sodium hydroxid used in titrating to the hydrogen-ion concentration P_H7 and $P_H8.3$ are obtained. Also, by subtracting the figures

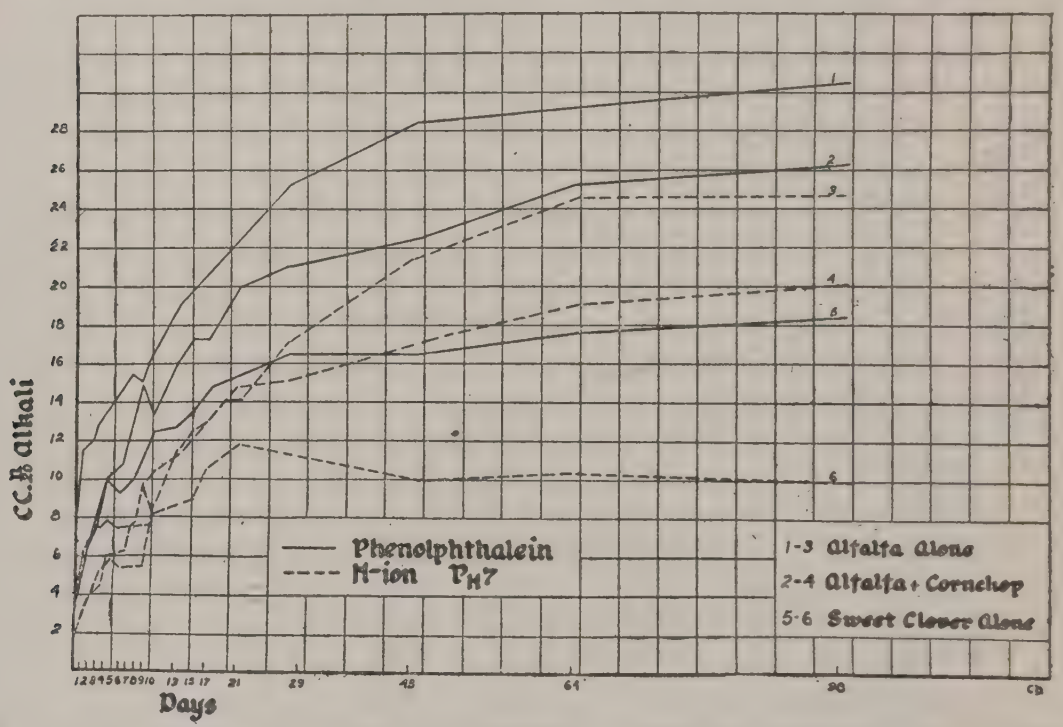

FIG. 3.—Graphs showing quantity, in cubic centimeters, of alkali used in alcoholic extract of silage-comparison of colorimetric titrations with phenolphthalein, and electrometric titrations to P_H7.

in the second part of Table IV from those in the third section the differences for the hydrogen-ion concentration $P_H8.3$ and $P_H9.3$ are obtained. The figures so obtained are found in Table V for both the water extract and the alcoholic extract.

The data in Table show that—

(1) The water extracts in comparison with the alcoholic extracts give substantially the same results (fig. 3–5).

(2) There is no significant change in the differences in the number of cubic centimeters of *N/20* sodium hydroxid used in titrating to a concentration, $P_H8.3$, in comparison with P_H7, as the silage matures—that is, the difference between the two points tends to be constant.

[1] SWANSON, C. O., and TAGUE, E. L. OP. CIT., p. 284.

TABLE V.—*Differences in the quantity, in cubic centimeters, of N/20 sodium hydroxid used to titrate to P_H 8.3 in comparison with P_H 7; also between P_H 9.3 and P_H 8.3*

FOR WATER EXTRACT OF SILAGE

Age of silage.	Alfalfa alone.		Sweet clover alone.		Sweet clover and cornchop.	
	Increase from P_H7 to P_H8.3.	Increase from P_H8.3 to P_H9.3.	Increase from P_H7 to P_H8.3.	Increase from P_H8.3 to P_H9.3.	Increase from P_H7 to P_H8.3.	Increase from P_H8.3 to P_H9.3.
Days.	*A*	*B*	*A*	*B*	*A*	*B*
0	1.0	1.8	1.9	3.4	1.7	1.9
2	2.6	3.6	2.4	6.2	3.3	4.7
2	4.7	7.8	2.1	5.5	2.4	5.3
3	5.0	7.9	3.5	5.4	3.7	5.1
4	3.6	6.6	3.5	7.0	3.5	3.7
6	4.4	6.7	2.7	4.8	3.0	5.4
7	4.6	5.4	.....	.....	.....	.....
8	3.7	5.9	2.3	5.0	2.8	5.7
10	4.1	8.9	2.5	5.2	3.0	5.4
13	3.5	7.8	2.4	5.5	2.6	7.7
15	3.0	8.7	2.9	7.8	2.7	9.8
17	3.1	12.8	2.7	6.8	2.7	9.6
21	6.5	10.2	3.3	7.5	4.6	8.9
29	5.8	11.7	3.0	9.6	3.5	7.5
43	7.0	10.1	3.8	7.0	4.0	7.3
64	7.5	11.0	3.5	7.1	4.9	7.1
98	4.5	12.6	1.6	6.8	4.5	5.1

FOR ALCOHOLIC EXTRACT OF SILAGE

0	2.1	3.1	1.3	2.0	1.1	1.9
1	3.2	5.8	1.7	3.6	2.4	3.6
2	3.5	6.3	2.2	4.4	1.8	4.5
3	3.3	6.9	2.2	4.7	2.4	5.2
4	3.4	7.2	2.4	6.0	2.2	5.6
6	3.8	7.9	2.3	5.1	2.6	6.2
7	3.6	7.8	.....	.....	.....	.....
8	3.6	10.3	2.3	5.7	2.8	5.8
10	3.6	9.2	2.8	6.3	2.7	6.6
13	3.7	9.5	2.8	6.0	2.1	7.2
15	3.8	9.2	2.7	6.7	2.6	6.4
17	3.8	10.3	3.0	7.2	2.6	7.6
21	3.9	10.1	3.2	7.4	3.1	7.8
29	3.5	11.9	3.1	8.3	3.1	8.5
43	4.4	12.3	2.9	8.1	3.4	7.9
64	3.8	12.4	3.0	8.8	4.0	8.8
98	4.5	13.3	2.9	8.5	3.6	9.0

(3) The quantity of *N/20* sodium hydroxid used in titrating to a concentration, P_H9.3, in comparison with the concentration, P_H8.3, increases as the silage matures. This increase is most notable in the silage from alfalfa alone. This larger and larger neutralization or absorption of the sodium hydroxid as the silage becomes older is probably due to the production of substances of the nature of proteoses or peptones. The proteins undergo a splitting process. When the *N/20* sodium hydroxid is

added, it combines with these hydrolytic protein products in larger and larger amounts as the hydrogen-ion concentration is reduced below P_H8.3 and more so when it approaches 9.3. If the titrations had been

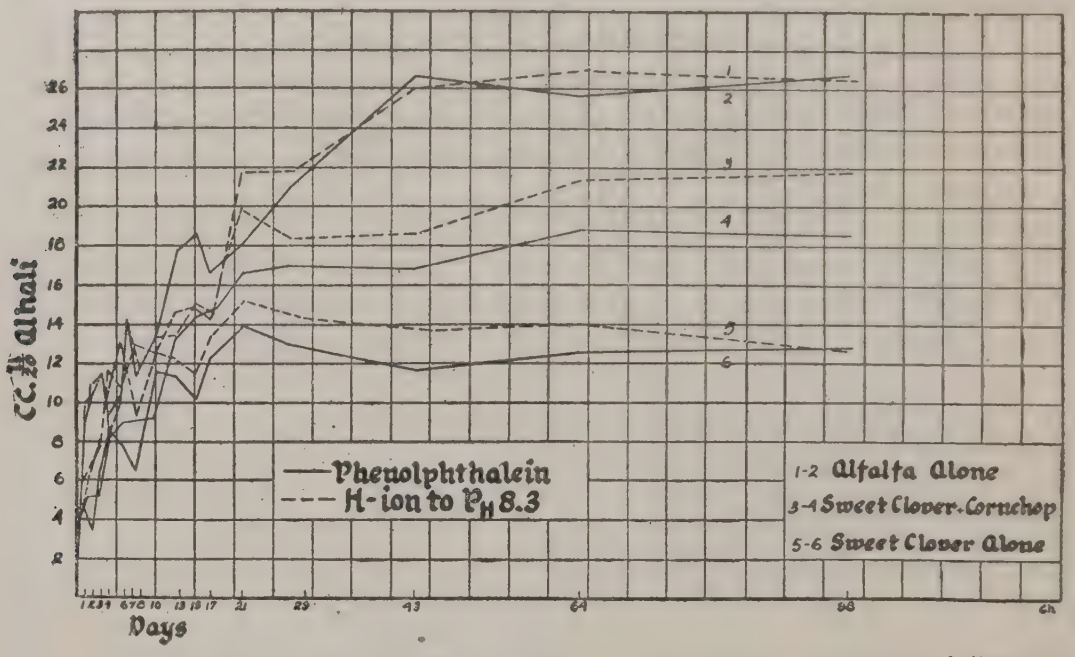

FIG. 4.—Graphs showing quantity, in cubic centimeters, of alkali used in water extract of silage; comparison of colorimetric titrations with phenolphthalein, and electrometric titrations to P_H8.3.

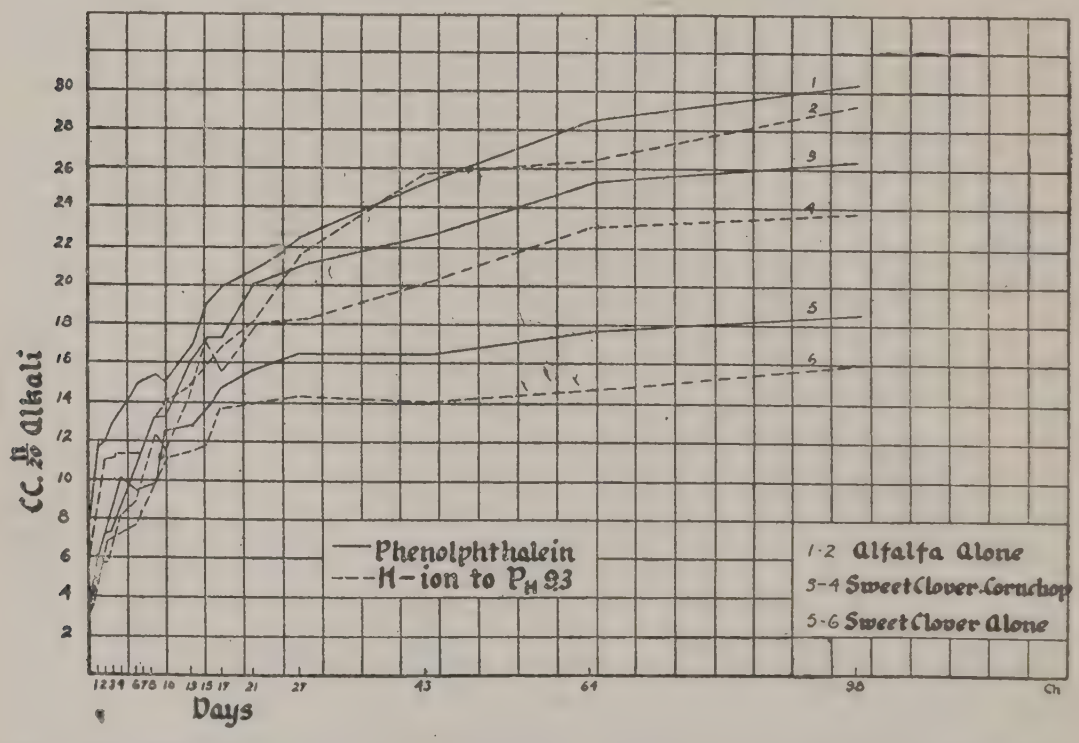

FIG. 5.—Graphs showing quantity, in cubic centimeters, of alkali used in alcoholic extract of silage; comparison of colorimetric titrations, with phenolphthalein, and electrometric titrations to P_H9.3.

carried to a concentration of P_H10 or lower, the differences obtained might have been even greater. Some work done in our laboratory on the hydrolysis of wheat substantiates this supposition.

(4) The fact that sweet clover shows smaller differences than alfalfa in these respects indicates that this protein hydrolysis takes place to a less extent in sweet clover than in alfalfa. This would mean that under practical conditions good silage is more readily made from sweet clover than from alfalfa. The addition of corn meal to sweet clover did not seem to have any distinct advantage. Silage from sweet clover alone was as good as that made from sweet clover and corn meal. With alfalfa the addition of corn meal was an advantage, as shown in our previous work.[1]

NITROGEN IN AMINO (NH_2) FORM

TITRATIONS WITH THE HYDROGEN ELECTRODE

It was stated that when making the determinations for acidity the hydrogen-ion concentration had been reduced to $P_H9.3$, or the theoretical points of color change for thymolphthalein, 25 cc. of the formaldehyde solution was added and the titration resumed till the hydrogen-ion concentration was again $P_H9.3$. This second titration should give figures for calculating the nitrogen in amino form. Whether this is the case is a question which we do not attempt to answer in the present paper. According to Sörensen,[2] who first elaborated the method, the titrations should first be carried to the neutral point for phenolphthalein, or $P_H8.3$, then the formaldehyde solution added, and the titration repeated till the point of color change for thymolphthalein, or $P_H9.3$ is reached. The number of cubic centimeters of *N/20* sodium hydroxid used in this second titration multiplied by 0.7 give the number of milligrams of titrable nitrogen in the mixture. If this is correct, the results of the electrometric titrations as we made them are too low for amino nitrogen when the number of cubic centimeters obtained in this last titration are multiplied by 0.7. But as both the $P_H8.3$ and the $P_H9.3$ points were determined in the titrations for acidity, a correction can be introduced. This is done in Table VI. In the A columns are given the number of cubic centimeters used in the second titration after the formalin (*HCHO*) was added to bring the P_H value again to the 9.3 point. In Table V is given the differences in titrations between titrating to $P_H8.3$ and $P_H9.3$. By adding these differences to the figures in the A columns of Table VI, the figures given in the B columns are obtained. These figures should be the same as if the titrations had been first carried to the $P_H8.3$ point in the acidity titration and then formalin added and then the titration resumed till the $P_H9.3$ point was reached. That this assumption is correct is substantiated by some work done by us on the changes which take place in

[1] SWANSON, C. O., and TAGUE, E. L. OP. CIT.

[2] SÖRENSEN, S. P. L. ENZYMSTUDIEN. *In* Biochem. Ztschr., Bd. 7, Heft ¼, p. 45–101. 1907. Allen's Commercial Organic Analysis . . . ed. 4, v. 8, p. 478, 488. Philadelphia, 1913.

the hydrolysis of wheat. By multiplying these figures in the B columns by 0.7 the number of milligrams of amino nitrogen are obtained.

TABLE VI.—*Quantity of nitrogen in titrable form as obtained by electrometric titration*

FOR WATER EXTRACT

Age of silage.	Alfalfa alone.			Sweet clover alone.			Sweet clover and cornmeal.		
	N/20 sodium hydroxid after adding formalin (cc.).	Quantity in A+column B, Table V, 2d sect. (cc.).	Mgm. amino nitrogen or cc. in B×0.7.	N/20 sodium hydroxid after adding formalin (cc.).	Quantity in A+column B, Table V, 2d sect. (cc.).	Mgm. amino nitrogen or cc. in B×0.7.	N/20 sodium hydroxid after adding formalin (cc.).	Quantity in A+column B, Table V, 2d sect. (cc.).	Mgm. amino nitrogen or cc. in B×0.7.
Days.	*A.*	*B*		*A*	*B*		*A*	*B*	
0	3.5	5.3	3.7	2.1	5.5	3.9	1.8	3.7	2.6
1	13.8	17.4	12.2	7.5	13.7	9.6	6.0	10.7	7.5
2	13.3	21.1	14.8	8.8	14.3	10.0	8.6	11.9	8.3
3	14.8	22.7	15.9	10.7	16.1	11.3	9.1	14.2	9.9
4	16.0	22.6	15.8	13.3	20.3	14.2	11.8	15.5	10.9
6	18.5	25.2	17.6	14.0	18.8	13.2	13.9	19.5	13.5
7	22.0	27.4	19.2	.....	.....	.....	.....	.....	.....
8	22.2	28.1	19.7	12.0	17.0	11.9	13.0	18.7	113.1
10	22.2	31.1	21.8	16.4	21.6	15.1	13.5	18.9	13.2
13	23.2	31.0	21.7	17.6	23.1	16.2	18.1	25.8	18.1
15	23.9	32.6	22.8	15.2	23.0	16.1	17.1	26.9	18.8
17	24.0	36.8	25.8	14.8	21.6	15.1	16.8	26.4	18.5
21	28.8	39.0	27.3	19.2	26.7	18.7	17.0	27.9	19.5
29	28.1	39.8	27.9	19.1	28.7	21.1	19.1	26.6	18.6
43	28.3	38.4	16.9	16.5	23.5	16.5	15.4	22.7	15.9
64	27.7	38.7	27.1	16.5	23.6	16.5	14.1	21.2	14.8
98	26.8	39.4	27.6	18.1	24.9	17.4	15.5	20.6	14.4

FOR ALCOHOLIC EXTRACT

0	3.1	6.2	4.3	2.0	4.0	2.8	2.0	3.9	2.7
1	9.0	14.8	10.4	5.0	8.6	6.0	4.7	8.3	5.8
2	13.1	19.4	13.6	6.4	10.8	7.6	6.2	10.7	7.5
3	12.9	19.8	13.9	7.8	12.5	8.8	6.4	11.6	8.1
4	12.5	19.7	13.8	9.3	15.7	11.0	8.8	14.4	10.1
6	14.1	22.0	15.4	8.3	13.4	9.4	8.2	14.4	10.1
7	13.3	21.1	14.8	.....	.....	.....	.....	.....	.....
8	14.8	25.1	17.6	9.2	14.9	10.4	10.1	15.9	11.1
10	13.8	23.0	16.1	10.3	16.6	11.6	9.6	16.2	11.3
13	16.5	26.0	18.2	11.6	17.6	12.3	11.4	18.6	13.0
15	16.7	25.9	18.1	12.0	18.7	13.1	13.2	19.6	13.7
17	17.4	27.7	19.4	12.0	19.2	13.4	12.6	20.2	14.1
21	16.5	26.6	18.6	12.6	20.0	14.0	14.2	22.0	15.4
29	19.3	21.2	21.8	13.5	21.8	15.3	13.8	22.3	15.6
43	22.6	34.9	24.4	13.2	21.3	14.9	14.3	22.2	15.5
64	23.0	35.4	24.8	14.6	23.4	16.4	13.8	22.6	15.8
98	23.0	36.3	25.4	16.1	14.6	17.2	16.0	25.0	17.5

The data in Table VI show that—

(1) The amount of amino nitrogen in the alcoholic extract and in the water extract is practically the same for the matured silage. In silage from 1 to about 2 weeks old the water extract shows the presence of a larger amount of amino nitrogen.

(2) The amount of amino nitrogen in the silage made from alfalfa is notably larger than that in the silage made from sweet clover.

(3) The amount of amino nitrogen in the silage made from sweet clover alone is practically the same as that in the silage made from sweet clover and corn meal. The addition of corn meal does not have any apparent influence on the amount of amino nitrogen produced.

TITRATIONS WITH INDICATORS

Titrations with indicators were made as follows: Twenty-five cc. of the extract were pipetted into a flask with 200 cc. of carbon-dioxid-free water, and 25 cc. of formalin added (1 part of 40 per cent formalin to 2 parts of carbon-dioxid-free water). This had been made neutral to thymolphthalein with sodium hydroxid. The mixture was well shaken and allowed to stand for 15 minutes, when it was titrated to a distant blue with *N*/20 sodium hydroxid, using 5 cc. of thymolphthalein as indicator. The total number of cubic centimeters obtained in this titration less the number of cubic centimeters used to obtain the acidity to phenolphthalein represents the number of cubic centimeters obtained in this titration less the number of cubic centimeters used to obtain the acidity to phenolphthalein represents the number of cubic centimeters due the titrable nitrogen. The figures as obtained are given in the A column of Table VII.

From the figures given in Table VII must be subtracted the figures for the acidity titration given in Table III. The remainder represents the amount of acidity due to the titrable nitrogen. These differences are given in the B columns of Table VII. These differences multiplied by 0.7 give the weight of titrable nitrogen. The results are given in the C columns. A comparison of the results in Table VI with those in VII shows that the results obtained by the electrometric and colorimetric methods are essentially the same. A direct comparison between the results on the water and the alcoholic extract, colorimetric titration, is given in Table VIII.

TABLE VII.—*Quantity of nitrogen in titrable form as obtained by colorimetric titration*

FOR WATER EXTRACT

Age of silage.	Alfalfa alone.			Sweet clover alone.			Sweet clover and corn meal.		
	Cc. of *N*/20 sodium hydroxid corrected for acidity to —		Mgm. of titrable nitrogen in B×0.7.	Cc. of *N*/20 sodium hydroxid corrected for acidity to —		Mgm. of titrable nitrogen in B×0.7.	Cc. of *N*/20 sodium hydroxid corrected for acidity to —		Mgm. of titrable nitrogen in B×0.7.
	Thymol.	Phenol.		Thymol.	Phenol.		Thymol.	Phenol.	
Days.	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
0	5.7	2.4	1.7	6.4	3.6	2.5	5.2	1.9	1.3
1	14.9	5.9	4.1	18.2	13.6	9.5	15.1	9.8	6.9
2	28.2	17.8	12.5	20.4	15.0	10.5	17.5	12.0	8.4
3	29.8	18.4	12.9	23.8	17.5	12.3	19.1	13.5	9.5
4	27.6	18.5	13.0	26.0	17.3	12.1	23.0	14.8	10.4
6	29.1	18.5	13.0	21.7	13.7	9.6	24.7	15.7	11.0
7	34.0	19.6	13.7	.....	.....	.....	.....	.....	.....
8	35.4	23.9	16.7	22.6	16.2	11.3	27.0	13.8	9.7
10	37.5	24.1	16.9	29.3	17.7	12.4	27.6	18.3	12.8
13	35.5	17.7	12.4	28.7	17.4	12.2	35.1	21.9	15.3
15	40.0	21.5	15.1	31.5	21.2	12.8	33.3	19.0	13.3
17	46.9	30.3	21.2	30.5	18.3	12.8	35.1	20.3	14.2
21	52.7	34.6	24.2	40.8	27.0	18.9	39.9	23.3	16.3
29	53.3	32.5	22.8	40.5	27.5	19.3	45.8	28.8	20.2
43	56.2	29.6	20.7	35.5	23.8	16.7	40.2	23.4	16.4
64	60.2	34.5	24.2	36.4	23.8	16.7	47.0	28.1	19.7
98	64.7	38.0	26.6	39.5	26.7	18.7	45.8	27.2	19.0

FOR ALCOHOLIC EXTRACT

0	14.1	6.4	4.5	7.7	4.1	2.9	8.2	4.4	3.1
1	27.2	15.5	10.9	14.3	8.2	5.7	14.6	8.9	6.2
2	31.4	19.4	13.6	18.5	11.3	7.9	17.3	10.4	7.3
3	32.5	19.6	13.7	20.9	12.2	8.5	17.8	10.2	7.1
4	32.0	18.5	13.0	24.9	14.8	10.4	22.8	12.8	9.0
6	34.7	19.8	13.9	21.9	12.5	8.8	24.8	13.9	9.7
7	36.0	20.6	14.4	.....	.....	.....	.....	.....	.....
8	39.7	24.7	17.3	23.9	14.0	9.8	29.4	14.6	10.2
10	41.5	24.6	17.2	27.0	14.5	10.2	27.2	14.0	9.8
13	44.2	25.2	17.7	29.7	16.9	11.8	32.6	16.5	11.6
15	45.5	25.6	17.9	30.6	17.0	11.9	33.9	16.6	11.6
17	46.9	26.1	18.3	33.4	18.7	13.1	36.0	18.7	13.1
21	47.9	25.5	17.9	34.0	18.5	13.0	40.7	20.7	14.5
29	55.7	30.5	21.4	35.2	18.8	13.2	41.1	20.1	14.1
43	60.0	31.6	22.1	38.7	22.2	15.5	44.7	22.3	15.6
64	64.0	33.6	23.5	39.1	21.4	15.0	44.4	19.2	13.4
98	.....	.....	.....	42.0	23.5	16.5	49.3	23.0	16.1

A comparison of the amount of amino nitrogen obtained by the Van Slyke method with the amounts obtained by the electrometric and colorimetric titrations was made. For this comparison bottles of matured alfalfa silage and sweet-clover silage were used. The Van Slyke determination was made in the usual way. The titrable nitrogen

was determined both by the electrometric and the colorimetric methods. The results from the Van Slyke method were very much larger, but we did not investigate the reasons for this. To make a thorough comparison of the Van Slyke method with the formol-titration method would probably require as extended an investigation as the one reported in this paper.

TABLE VIII.—*Comparison of amount of titrable nitrogen stated as milligrams per 5 gm. of silage in alcoholic and water extracts, colorimetric titration*

Age of silage.	Alfalfa alone.		Sweet clover alone.		Sweet clover and corn meal.	
	Water.	Alcohol.	Water.	Alcohol.	Water.	Alcohol.
Days.						
0	1.7	4.5	2.5	2.9	1.3	3.1
1	4.1	10.9	9.5	5.7	6.9	6.2
2	12.5	13.6	10.5	7.9	8.4	7.3
3	12.9	13.7	12.3	8.5	9.5	7.1
4	13.0	13.0	12.1	10.4	10.4	9.0
6	13.0	13.9	9.6	8.8	11.0	9.7
7	13.7	14.4	.....	.....	.....	.....
8	16.7	17.3	11.3	9.8	9.7	10.2
10	16.9	17.2	12.4	10.2	12.8	9.8
13	12.4	17.6	12.2	11.8	15.3	11.6
15	15.1	17.9	12.8	11.9	13.3	11.6
17	21.2	18.3	12.8	13.1	14.2	13.1
21	24.2	17.9	18.9	13.0	16.3	14.5
29	22.8	21.4	19.3	13.2	20.2	14.1
43	20.7	22.1	16.7	15.5	16.4	15.6
64	24.2	23.5	16.7	15.0	19.7	13.4
98	26.6	.....	18.7	16.5	19.0	16.1

TOTAL, STUTZER'S, OR ALBUMINOID, AND AMID NITROGEN IN SILAGE, KJELDAHL METHOD

The total and Stutzer's nitrogen, sometimes called "albuminoid nitrogen," were determined on the samples from the moisture determinations. The result stated as milligrams per 5 gm. of silage are given in Table IX.

The results given in Table IX show that—

(1) In silage from alfalfa alone the nitrogen in amid [1] form is approximately one-half of the total. In silage from sweet clover alone it is a little more than one-half of the total, and when corn meal is added, the proportion is somewhat smaller.

[1] The term "amid" is used simply to designate the difference between the total and albuminoid nitrogen as determined by Stutzer's method (WILEY, H. W., ED. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agri. Bur. Chem. Bul. 107 (rev.), p. 38, 1908. Reprinted, 1912.

(2) The nitrogen in amid form as determined by this method does not show the same degree of progressive increase as the nitrogen determined by titration, as shown in Table VIII. The drying of the sample may have something to do with this. That the process of drying may influence the form of nitrogen in forage material is shown by investigations now in progress at this laboratory.

(3) The amount of nitrogen in amid form in matured silage is a little larger as determined by Stutzer's method than by the formaldehyde titration method, but the differences are not large. Compare Tables VI and VII with Table IX.

TABLE IX.—*Total nitrogen, Stutzer's nitrogen, and amid nitrogen in silage*

[Results are expressed as milligrams per 5 gms. of silage.]

Age of silage.	Alfalfa alone.			Sweet clover alone.			Sweet clover and corn meal.		
	Total nitrogen.	Stutzer's nitrogen.	Amid nitrogen.	Total nitrogen.	Stutzer's nitrogen.	Amid nitrogen.	Total nitrogen.	Stutzer's nitrogen.	Amid nitrogen.
Days.									
1	58.0	29.0	29.0	33.5	21.5	12.0	42.0	27.0	15.0
2	56.5	29.5	27.0	36.0	22.0	14.0	41.5	26.5	15.0
3	60.0	32.0	28.0	36.5	20.0	16.5	40.0	25.5	14.5
4	58.5	29.0	29.5	36.5	20.0	16.5	35.0	24.5	10.5
6	70.5	25.0	45.5	35.5	19.5	16.0	43.0	26.0	17.0
7	52.5	26.0	26.5	.....	.....	.....	.....	.....	.....
8	49.5	26.5	23.0	35.0	19.0	16.0	38.0	23.5	14.5
10	52.0	25.5	26.5	33.5	16.5	17.0	39.5	23.5	16.0
13	52.0	24.5	27.5	32.5	15.5	17.0	42.5	24.0	18.5
15	48.5	22.5	26.0	30.0	15.0	15.0	41.5	23.0	18.5
17	52.0	24.0	28.0	37.0	16.5	20.5	40.5	21.5	19.0
21	53.5	27.5	26.0	36.0	15.0	21.0	41.0	22.0	19.0
29	59.0	26.5	32.5	34.0	14.0	20.0	39.0	21.5	17.5
43	54.5	30.0	24.5	34.0	16.0	18.0	35.5	19.5	16.0
64	54.0	25.5	28.5	29.5	14.0	15.5	35.5	20.0	15.5
98	51.0	24.0	27.0	32.0	14.0	18.0	37.0	19.0	18.0

NITROGEN IN WATER AND ALCOHOLIC EXTRACTS OF SILAGE, KJELDAHL METHOD

The water and alcoholic extracts of silage were used for the determination of nitrogen, Kjeldahl method, both total and that not precipitated by phosphotungstic acid. The results stated in milligrams per 5 grams of silage are given in Table X.

The results given in Table X show that—

(1) The water and the alcoholic extracts gave practically the same amount of total nitrogen, showing that the solvent action of 50 per sent alcohol is not different from that of water.

(2) The soluble nitrogen is approximately two-thirds of the total in the silage made from alfalfa alone and from sweet clover alone. When corn meal was added to sweet clover, the proportion of water-soluble to total nitrogen was a little more than one-half. Compare Table IX with Table X.

(3) In the water extract the nitrogen not precipitated by phosphotungstic acid is approximately three-fourths of the total soluble nitrogen. In the alcoholic extract the nitrogen precipitated by phosphotungstic acid is practically the same in the amount as the total soluble in 50 per cent alcohol.

(4) Thus, while the total solubility is not much different in the water and alcoholic extracts, the nature of the solution is different as measured by the precipitating action of phosphotungstic acid. This is either due to the solubility of the protein phosphotungstate in the alcohol or to the fact that the ionization is such that the precipitate will not form when the phosphotungstic acid is added.

TABLE X.—*Total nitrogen and nitrogen not precipitated by phosphotungstic acid in water and alcoholic extracts of silage*

[Results expressed as milligrams of nitrogen from 5 gm. of silage.]

Age of silage.	Alfalfa alone.				Sweet clover alone.				Sweet clover and corn meal.			
	Water extract.		Alcoholic extract.		Water extract.		Alcoholic extract.		Water extract.		Alcoholic extract.	
	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.	Total nitrogen.	Nitrogen not precipitated by phosphotungstic acid.
Days.												
0	8.5	3.0	.....	.....	7.0	4.5	.....	.....	11.0	4.5	.....	.....
1	26.0	15.0	23.0	24.5	19.0	10.5	15.5	14.5	17.0	9.0	16.5	14.5
2	30.0	20.5	26.0	28.0	7.5	12.0	15.0	16.5	16.0	11.0	17.0	15.5
3	29.0	20.0	28.0	29.5	19.0	12.0	18.0	18.5	18.0	10.5	18.0	16.0
4	27.5	16.5	26.5	28.5	20.0	14.5	20.0	20.5	17.0	12.0	19.0	19.0
6	26.5	21.0	29.0	30.5	18.5	13.5	17.5	18.0	20.0	15.0	21.5	19.0
7	30.0	24.0	26.0	28.0	.....	.....	.....	.....	.....	.....	.....	.....
8	30.5	19.0	29.5	31.0	15.0	14.0	18.0	19.0	19.5	14.0	21.5	19.5
10	30.5	23.5	30.0	31.0	22.0	17.5	20.0	20.5	20.5	14.5	22.0	20.0
13	29.0	22.0	31.0	34.0	21.0	15.5	21.5	21.0	22.0	16.0	24.5	22.5
15	31.0	22.5	30.0	31.0	21.0	17.5	20.5	21.0	22.5	17.0	24.5	22.5
17	34.0	31.5	31.5	32.5	31.5	16.5	21.0	21.5	21.5	16.5	24.5	22.5
21	33.5	24.5	31.0	32.0	22.5	16.0	22.0	22.5	23.5	16.0	26.0	25.0
29	35.0	24.5	35.0	36.5	23.0	17.5	22.5	24.0	25.0	18.0	26.0	24.5
43	38.0	27.0	36.5	31.5	22.5	16.0	21.5	23.0	23.5	17.5	25.5	24.5
64	37.0	28.5	38.0	42.0	21.0	14.5	22.5	26.5	23.0	17.0	14.0	26.5
98	34.5	27.5	36.5	38.0	20.5	12.5	25.0	24.0	24.0	19.5	27.5	26.5

SUMMARY

(1) In this paper have been presented the results of making determinations both by the colorimetric and electrometric methods in the water and alcoholic extracts of silage made from alfalfa alone, from sweet clover alone, and from sweet clover plus corn meal. These extracts were also used for the determinations of nitrogen in amino form by the colorimetric and electrometric methods. Total nitrogen and albuminoid, or Stutzer's nitrogen, were also determined on these extracts. Quart milk bottles were used as containers for the silage. A number of bottles of each kind of silage were made and these were opened at increasingly longer intervals of time. In this way the progressive chemical changes were traced.

(2) The weight of the bottles just after filling and when opened showed that the losses were approximately 1 per cent.

(3) The acidity of the alcoholic extracts of the three kinds of silage was greater than that of the water extract when the titration was made to the point of color change for phenolphthalein. When the electrometric method was used and the titration was made to a hydrogen-ion concentration of $P_H 8.3$, the point of color change for phenolphthalein, there was no significant difference between the results obtained on the water extract and that of the alcoholic extract. The greater values obtained on the alcoholic extracts with the colorimetric method are probably due to the highly colored material extracted by the alcohol. These mask the end point.

(4) Most of the acidity is developed in the first 15 days.

(5) Adding corn meal to sweet clover increases the amount of acidity in the resulting silage.

(6) The amount of amino nitrogen is practically the same in the water and the alcoholic extracts. The amount of amino nitrogen in silage made from alfalfa alone is notably larger than that made from sweet clover alone. The addition of corn meal to sweet clover has no influence on the amount of amino nitrogen developed.

(7) The amount of nitrogen in amid form as determined by Stutzer's method was a little larger than the amount of nitrogen in amino form as determined by the formaldehyde method, but the differences were not large. The nitrogen in amid form was approximately one-half of the total.

(8) Approximately two-thirds of the total nitrogen in silage is soluble in water and 50 per cent alcohol, the solvent action of the two being nearly the same.

(9) From the various data presented, it appears that silage can be made from sweet clover alone with less difficulty than from alfalfa alone.

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EFFECT OF HYDROCYANIC ACID GAS UNDER VACUUM CONDITIONS ON SUBTERRANEAN LARVÆ

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INTRODUCTION

The advisability of fumigating all imported nursery stock at the port of arrival as a requirement for entry has been under consideration for the past five or six years. It is generally recognized that hydrocyanic acid gas under proper temperature conditions is effective against practically all insects likely to be introduced, provided these pests are neither hermetically sealed in the tissues of the plants nor protected by the soil around the roots.

Under normal conditions from five to seven million ornamental plants, such as azaleas, rhododendrons, arbutus, conifers, box hedges, ferns, etc., are introduced annually with balls of earth around their roots. Needless to say, it is impossible satisfactorily to inspect and safeguard plants of this type without removing the soil from the roots. Inasmuch as practical horticulturists have strongly advised against this procedure on account of its effect on the plants, the only safe course appeared to be fumigation with hydrocyanic acid gas under vacuum conditions. With this end in view, experiments, listed in Table I, were conducted in which various dosages and exposures were used, in order that the effectiveness and the degree of safety which might be obtained by fumigating all imported plants having balls of earth around their roots could be determined.

EXPERIMENTAL PROCEDURE

Owing to the variable amounts of water in the soil surrounding the roots of balled plants at the time of arrival, it was considered advisable to fumigate subterranean larvæ buried at various depths in dry, moist and soaked soil.[1]

The water content of the moist and of the soaked soil compared favorably with the condition of the soil around the balls of plants at the time of arrival. Very few plants would survive shipment in soil as free from moisture as that used in the dry experiment. Furthermore, the soil

[1] The soil used consisted of 1 part of manure, 1 part of peat, and 1 part of loam.

employed was not as compact as is usually the case with the average balled plant.

Through the kindness of Messrs. J. A. Hyslop, J. J. Davis, and W. O. Ellis, of the Bureau of Entomology, United States Department of Agriculture, larvæ of the wheat wireworm (*Agriotes mancus* Say), green June beetle (*Cotinus nitida* Linnaeus), white grub (*Lachnosterna* sp.), and *Popillia japonica* Newman were obtained and fumigated under the following conditions:

All larvæ were exposed to the gas in potting soil, previously described, contained in 3-inch flowerpots, with the exception of those used in experiments 8, 9, and 10 (Table I), in which case 5-inch pots were employed. The position of the larvæ varied, being from 1 to 3 inches from the surface of the soil.

After the larvæ had been placed in the soil, the pots were transferred to the fumigation chamber, the door closed, and the required vacuum produced. At this point the gas was generated and allowed to pass immediately to the fumigation chamber. At the completion of the required exposure the gas was removed from the fumigation chamber by drawing a 25-inch vacuum.

In experiments 2 and 3 the larvæ were exposed to the gas for the entire period in a vacuum; in experiment 4 they were exposed to the gas for 30 minutes in a vacuum, and 30 minutes under normal atmospheric pressure; and in experiment 5 they were exposed to the gas in a vacuum for 30 minutes, followed by a pressure of 10 pounds for 30 minutes. With the exceptions noted, all experiments were conducted with a preliminary vacuum of 26 inches for 15 minutes, with exposures under normal atmospheric conditions varying from 1½ to 2 hours.

CHEMICALS EMPLOYED IN THE GENERATION OF HYDROCYANIC-ACID GAS

Sodium cyanid guaranteed to contain not less than 51 per cent of cyanogen and commercial sulphuric acid (about 1.84 sp. gr., or 66° Baumé) were used in all experiments. The cyanid was used in solution prepared by dissolving 4 pounds of sodium cyanid in 1 gallon of water. The formula employed was as follows:

Sodium cyanid in solution	ounces	2½
Sulphuric acid	ounce	1
Water	ounce	1

RESULTS OF THE EXPERIMENTS

Taken collectively the results given in Table I show that the killing of 100 per cent can not be depended on where the larvæ are in balls of earth around the roots of plants, providing a dosage is used which will not injure the stock. Particularly is this true in the case of moist and soaked soil.

TABLE I.—*Results of experiments in the fumigation of subterranean insects with hydrocyanic-acid gas under vacuum conditions*

Experiment No.	Insects fumigated.	Number of larvæ in soil.			Rate per 100 cubic feet.	Exposure.	Temperature.	Results in—		
		Dry.	Moist.	Soaked.				Dry soil.	Moist soil.	Soaked soil.
					Ounces.		°*F.*			
1	*Lachnosterna* sp.	3	3	3	½	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	78	3 dead.	3 dead.	1 dead, 2 alive.
1	*Agriotes mancus*	3	3	3	½	do		do	do	3 alive.
2	do	4	4	4	1	1 hour, vacuum 26 inches	84	4 alive.	4 alive.	4 alive.
3	do	5	5	4	1	1 hour, vacuum at start 27 inches; vacuum at completion 23 inches.	84	5 alive.	5 alive.	Do.
4	*Lachnosterna* sp.	5	5	5	1	½ hour, vacuum 27 inches; ½ hour, normal atmospheric pressure.	75	5 dead.	do	1 dead, 4 alive.
4	*Agriotes mancus*	5	5	5	1	do		1 dead, 4 alive.	do	5 alive.
5	*Lachnosterna* sp.	3	3	3	1	½ hour, vacuum 27 inches; ½ hour, pressure 10 pounds.	69	1 dead, 2 alive.	2 dead, 1 alive.	3 alive.
5	*Agriotes mancus*	3	3	3	1	do		3 alive.	3 alive.	Do.
6	*Lachnosterna* sp.	3	3	3	1	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	78	3 dead	3 dead.	[a]3 dead.
6	*Agriotes mancus*	3	3	3	1	do		do	do	3 dead.
7	*Lachnosterna* sp.	3	3	3	1	do	74	do	do	1 dead, 2 alive.
7	*Agriotes mancus*	3	3	3	1	do		do	do	Do.
7	*Cotinus nitida*	3	3	3	1	do		do	do	2 dead, 1 alive.
8	*Popillia japonica*	5	5	5	1	do	80	5 dead.	5 dead.	5 alive.
9	do			4	1	do	73			2 dead, 2 alive.
10	*Lachnosterna* sp.			3	1	¼ hour, vacuum 26 inches; 2 hours, normal atmospheric pressure.				3 alive.
10	*Agriotes mancus*			3	1	do	80			Do.
10	*Cotinus nitida*			3	1	do				2 dead, 1 alive.
11	*Lachnosterna* sp.			3	1½	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	85			Do.
11	*Agriotes mancus*			3	1½	do				Do.
11	*Cotinus nitida*			3	1½	do				3 dead.
12	*Popillia japonica*			4	3	do	72			4 alive.

[a] Although all the larvæ were dead, the result is not conclusive, since the larvæ when examined were found to be near the top and side of the pot.

As shown in experiments 2 and 3, satisfactory results are not obtained where the gas is held in the presence of a partial vacuum throughout the entire exposure. It has also been proved that a 30-minute exposure of gas in the presence of a partial vacuum and a 30-minute exposure under normal atmospheric conditions are not effective. The addition of 10 pounds' pressure for 30 minutes in lieu of normal atmospheric pressure yielded practically the same results. It is obvious from Table I that the most satisfactory results were invariably secured where a 15-minute preliminary vacuum was followed by an exposure of one or more hours under normal atmospheric conditions. Especially was this true in the case of dry and moist soil used in experiments 1, 6, 7, and 8, where 100 per cent of the larvæ of *Cotinus nitida*, *Agriotes mancus*, *Popillia japonica*, and *Lachnosterna* sp. were killed. In the soaked-soil tests, however, the results were unsatisfactory.

While there was no notable difference in the resistance to the gas by the various larvæ used, it was apparent that *Popillia japonica* was the most difficult to kill, whereas *Cotinus nitida* was the most susceptible to fumigation.

SUMMARY

(1) The effectiveness of the hydrocyanic-acid gas under the vacuum process is influenced by the water content of the soil.

(2) The death of 100 per cent was not obtained with larvæ in soaked soil at dosages ranging from ½ ounce to 3 ounces per 100 cubic feet of space.

(3) Eliminating the soaked-soil tests, by far the best results were secured where a preliminary 15-inch vacuum preceded an exposure of one and a half hours under normal atmospheric conditions.

(4) Hydrocyanic-acid gas in the presence of a 26-inch vacuum throughout the entire exposure gave negative results with a dosage of one ounce of sodium cyanid per 100 cubic feet and an exposure of 1 hour. An exposure of the gas for one-half hour under 10 pounds' pressure, following a half-hour exposure to a 27-inch vacuum, yielded very indifferent results.

(5) With our present knowledge of vacuum fumigation with hydrocyanic-acid gas, a dosage exceeding 1 ounce of sodium cyanid per 100 cubic feet of space with an exposure of 1½ hours is not recommended for plants in foliage. Inasmuch as all larvæ in soaked soil were not killed with dosages varying from ½ ounce to 3 ounces per 100 cubic feet of space, fumigation at the port of entry with a dosage which will not injure the plants can not prevent the introduction and establishment of all subterranean pests.

CATALASE AND OXIDASE CONTENT OF SEEDS IN RELATION TO THEIR DORMANCY, AGE, VITALITY, AND RESPIRATION

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INTRODUCTION

For some years the Seed-Testing Laboratories of the Bureau of Plant Industry has been using daily alternating temperatures for the germination of certain seeds. In these alternations the lower temperature is used about 18 hours and the higher temperature about 6 hours of the day. Alternating temperatures are found especially favorable for the germination of the seeds of certain grasses: Johnson grass, *Holcus halepensis* L. (*Sorghum halepensis* Pois.); bluegrass, *Poa pratensis;* and Bermuda grass, *Capriola dactylon* (L.) Kuntze. For Johnson grass 30° C. for 18 hours and 45° C. for 6 hours of the day have been found to be the most favorable alternation. Sudan grass, *Holcus halepensis sudanensis* (Piper) Hitchc. (*Andropogon halepensis sudanensis* Piper), which is closely related to Johnson grass and very similar to it in most respects, germinates readily at constant temperatures.

During the last three years (1916–1918) an extensive physiological study of the behavior of the seeds of Johnson grass and Sudan grass has been made in order to get some light on the reasons for the difference in the requirements of the two regarding alternate temperatures for germination. Incidentally such a study has thrown much light upon delayed germination and the physiology of germination in general. Naturally seeds of several other grasses and of a number of other families have been drawn into the investigation. The present paper reports one phase of this extensive comparative study of the physiology of germination.

MECHANICS OF DORMANCY

A brief statement of the mechanics of dormancy in seeds can profitably be made at this point, since it will throw light on experiments to be reported later. There are two general means by which seeds are rendered dormant even when they have morphologically mature embryos and have all the external conditions necessary for germination.

[1] The writers are under great obligation to the following organizations for supplies of seeds used in this work; To the Office of Forage Crop Investigations for Johnson grass and Sudan grass seeds of various ages, together with data as to time and place of collection; to the Seed-Testing Laboratories for several samples of 23-year-old seeds of *Amaranthus retroflexus;* and to the botanical staff of the State College of Washington for the collections of various species of Amaranthus made at Pullman, Wash.

(1) In one type the embryo is dormant (incapable of growth when it is naked and furnished ordinary germination conditions) and must go through some very fundamental changes preliminary to growth. The changes generally require considerable time—weeks or months. Some experiments already published (*15*, *19*, *20*)[1] and many unpublished experiments made at the Hull Botanical Laboratory by Mr. W. E. Davis and by Mr. R. C. Rose, together with experiments reported in this paper, show the optimum condition for after-ripening of dormant embryos, as well as some of the changes that occur in the embryo during after-ripening. The seeds of this type that have been studied in some detail are: Various species of Crataegus, peach (*Amygdalus persica*), *Ambrosia trifida*, *A. artemisiaefolia*, and basswood (*Tilia americana*). The optimum conditions for the changes seem to be a temperature of about 5° C., with a good oxygen and water supply. The removal of the carpels or other coat structures hastens the process markedly, and it does so, in part at least, by increasing the water and oxygen supply. The following changes have been noted as after-ripening progresses: Continuous rise in the vigor of the resulting seedling, as manifested by rate of growth and resistance to fungal attack; increase in the amount of water absorbed; increase in total acid; slight (due to buffers) but evident increase of H^+ concentration; increase in catalase activity; and increase in oxidase activity, at least as manifested by action on guaiacum and on chromogens already in the plant.

(2) In the second type of dormancy inclosing structures prevent growth of the embryo by holding out some growth factor or holding in some growth inhibitor. In hard seeds water is entirely excluded. In Amaranthus and Alisma (*15*, *16*) an initial rapid water absorption ceases before the imbibitional and osmotic forces of the embryo are satisfied, because the swelling of the seed contents is not sufficient to break the seed coats. In many seeds the coats reduce the oxygen supply below the necessary minimum for germination (*15*).

If freshly harvested seeds of Johnson grass are kept in a germinator at 20° C., they will remain dormant for more than a year and probably for many years. Under this condition they go through a change by which they become less capable of germination at higher constant temperatures (25° or 30° C.) than fresh seeds and very much less so than dry stored seeds of the same collection. The senior writer (*15*) has spoken of this deepened dormancy produced in a germinator furnishing conditions unfavorable for germination as "second dormancy" and has pointed out its rather general occurrence as reported in the literature. Johnson grass seeds in dry storage gradually after-ripen, so that their germination improves both at alternate and constant temperatures.

The dormancy in seeds of Johnson grass is imposed by structures inclosing the embryo (scales, pericarp, and tegmen), for removal of

[1] Reference is made by number (italic) to "Literature cited," p. 172–174.

these structures leads to very prompt and practically complete germination in fresh seeds, after-ripened seeds, and seeds in secondary dormancy.[2] The seedlings resulting in every case are about equally vigorous. This indicates that the embryo itself is not dormant. As yet how the inclosing structures enforce dormancy is not known. It is not by the complete exclusion of water, for the intact seeds absorb water rather readily; nor through reduction of the oxygen supply, for these seeds are indifferent to variations in oxygen pressures varying from one-fifth to five-fold that of the atmosphere; nor to narcotic action of carbon dioxid held in by the coats, as Kidd (*28*) has assumed to be the case for all dormant seeds that absorb water readily, for carbon dioxid in high partial pressures is a good forcing agent for dormant seeds of Johnson grass.

CATALASE IN SEEDS

Catalase is an enzym capable of splitting hydrogen peroxid into water and oxygen. It is universally present in living matter and was supposed to be a property of all enzyms until Loew (*30*) showed it to be a distinct body. There is some question arising as to its real enzymic nature (*1*, *2*).

Its function in the organism is not known. Loew believed that in aerobes it protected the organism against the accumulation of hydrogen peroxid produced in respiration. In anaerobes he assumed that it loosened chemical affinities, aiding splittings, oxidations, and reductions. Others have assigned it protective action against excessive oxidations in the organism by organic peroxids or even an essential part in respiration (*27*, *p. 138-140*). Whatever its function Zieger (*37*) has shown some correlation between catalase content and metabolism in animals, and several workers (*2*, *12*, *13*, *29*, *32*) have shown a rather close correlation between respiratory intensity and catalase activity.

EXPERIMENTAL METHODS

The catalase activity was determined by an apparatus similar to that used by Appleman (*1*). A given weight of seed material, after being ground in a mortar and worked through a piece of bolting cloth of desirable mesh, was shaken up in the bottle of the apparatus with 5 cc. of distilled water. Then 5 cc. of hydrogen peroxid, rendered neutral to phenolphthalein by the addition of *N/10* sodium hydroxid, was placed in the dropping funnel and the bottle and dropping funnel lowered into a water bath at 25° C. After the apparatus with its contents had reached the temperature of the water bath the hydrogen peroxid was dropped into the plant emulsion and the mixture continuously shaken. The

[2] One of the best ways to remove these structures is to pick the caryopses out of the scales and treat them in the air-dry condition for two or three minutes with concentrated sulphuric acid. This treatment is followed by thorough washing, first with a 5 per cent solution of sodium hydrogen carbonate and later with distilled water. In the last washing care should be taken to rub away all the carbonized material.

amount of oxygen delivered in any given time was read in the gas burette. The amount of ground material used for each experiment varied from 0.5 to 0.025 gm., depending upon the catalase activity of the tissue. In general 0.14 gm. gave a delivery rate suitable to the size of the apparatus, and except when otherwise stated that is the amount used for each determination in this paper. Bolting cloth of 70 to 80 mesh to the inch was found most desirable. This degree of pulverizing gave the maximum activity for Johnson grass, although finer grinding (100 mesh to the inch) gave somewhat higher activity for the clover.

Dioxogen (H_2O_2 12 V.), of the Oakland Chemical Co., was used almost exclusively in these experiments, but two other brands of hydrogen peroxid were also tried, peroxid of hydrogen, 3.10 per cent, of the Oakland Chemical Co., and hydrogen peroxid, 2.7 per cent, of the J. T Baker Chemical Co. The Bureau of Chemistry furnished the following pharmacopeial analyses of these peroxids:

Peroxid of hydrogen (Oakland Chemical Co.)

Available H_2O_2	2.77 per cent.
Nonvolatile matter in 20 cc	0.021 gm.
Acidity, 25 cc	1.4 cc. *N/10*.
No preservative detected.	

Hydrogen peroxid, C. P. (J. T. Baker Chemical Co.)

Available H_2O_2	2.92 per cent.
Nonvolatile matter, 20 cc	0.036 gm.
Acidity, 25 cc	1.77 cc. *N/10*
Acetanilid (declared 1 part in 7,000)	1 part in 18,000.

Dioxogen (Oakland Chemical Co.)

Available H_2O_2	3.63 per cent.
Nonvolatile matter, 20 cc	0.011 gm.
Acidity, 25 cc	0.9 cc. *N/10*.
No preservative detected.	

It is interesting to compare the percentage of H_2O_2 found in these peroxids by the pharmacopeial method with the percentages calculated from the amount of gas delivered upon adding an excess of seed catalase. In the determinations with catalase 2 gm. of the unneutralized peroxid was diluted with 3 cc. of distilled water, and to this was added an emulsion of 1.5 gm. of powdered crimson-clover seeds in 5 cc. of water. The gas delivered was reduced to standard pressure and temperature and its weight calculated upon the assumption that it was all oxygen. From this the percentage of H_2O_2 in the peroxids was then calculated. The values given in every case are the average of two or more closely agreeing determinations. These percentages are shown in Table I.

It is seen that the catalase determinations are somewhat larger in every case and that the percentage excess is about constant. This discrepancy is probably due to a consistent error in one or the other of

the series of determinations, but the possibility remains that the catalase may split some of the H_2O_2 into hydrogen and oxygen. On the basis of the first and the more likely assumption it seems probable that catalase gives a complete decomposition of H_2O_2.

The biologist needs to know the concentration of the hydrogen peroxids he uses, whether they are used as sterilizing agents, forcing agents for dormant seeds, or for catalase determinations. He will find the catalase method sufficiently accurate and easily run with materials at hand. The determination can be made without neutralizing the acidity of the peroxids, even when it is rather high. The great excess of the plant powder used (probably acting as a buffer), along with the dilution of the peroxids, sufficiently counteract the inhibiting effects of the acids to give complete decomposition.

We have found dioxogen a desirable brand of hydrogen peroxid for catalase determinations and for use as a forcing agent for dormant seeds. It bears a rather high percentage of H_2O_2. The concentration runs almost constant for different bottles of the same lot (date cut into

TABLE I.—*Percentage of H_2O_2 in hydrogen peroxids*

Source of material.	Method of determination.	
	Excess of catalase.	Pharmacopeial.
Peroxid of hydrogen, Oakland Chemical Co.	2.77	2.68
Hydrogen peroxid, J. T. Baker Chemical Co.	3.02	2.92
Dioxogen, Oakland Chemical Co.	3.75	3.62

the label) and for bottles of different lots so far as our examinations have gone. Its acidity is also low and about constant.

Before using a bottle of dioxogen we always determined the percentage of H_2O_2 in it. We also neutralized portions of it to phenolphthalein as they were to be used. The 5 cc. employed in each catalase determination is capable of delivering about 65 cc. of oxygen at the temperature of the experiment. The concentration of catalase and time was so adjusted that in general not more than one-half the oxygen was delivered in an experiment, thus giving a great excess of hydrogen peroxid.

EXPERIMENTAL WORK

EFFECT OF ACIDITY

Various workers (*1*, *30*) have mentioned the sensitiveness of catalase to acids and have pointed out the fact that maximum activity and minimum destruction occur in a neutral or slightly alkaline medium.

Appleman (*1*) seems to consider the plant material the main source of destructive acids. In our work with seeds the hydrogen peroxids are the main source of injurious acids. Without neutralization the two more acid hydrogen peroxids mentioned above give (with Johnson grass) only about one-third as much catalase activity as does the less acid dioxogen. When all these peroxids are neutralized or an excess of calcium carbonate is used, the three give more nearly equal catalase activity. The more acid ones are still somewhat below the dioxogen, due either to lower concentration or other inhibitors. Table II shows the effect of the reaction of the dioxogen upon the catalase activity of Johnson grass.

TABLE II.—*Catalase activity as modified by the reaction of the dioxogen*

[Johnson grass dry-stored 1 year. 70-mesh bolting cloth. 0.1 gm. of meal. 0.9 cc. of *N*/10 *sodium hydroxid* needed to neutralize 25 cc. of dioxogen to phenolphthalein]

Amount of *N*/10 sodium hydroxid added.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
One-half amount needed to neutralize	4.1	7.5	9.9	13.9
Full amount needed to neutralize	4.2	7.5	10.0	14.1
Twice amount needed to neutralize	4.3	7.8	10.0	13.9
Three times amount needed to neutralize	4.1	7.6	9.7	12.8
None	1.9	4.3	5.6	7.9
Excess of calcium carbonate	4.1	7.8	10.2	13.9

Table II shows that the natural acidity of dioxogen reduces greatly the catalase activity of Johnson grass, but that an addition of sodium hydroxid from one-half of the amount needed for neutralization to phenolphthalein to twice the amount needed for such neutralization gives maximum activity. Dioxogen that is half neutralized to phenolphthalein is still acid to neutral red. As is well known, neutral red turns practically at the neutral point ($H^+ = 10^{-7}$), while phenolphthalein turns at a point that is distinctly basic ($H^+ = 10^{-9}$). The filtrate from the dioxogen-catalase mixture (the dioxogen being neutral to phenolphthalein) in the above experiments is basic to neutral red, while the filtrate from the emulsion of seed material is acid to neutral red. From all the facts here reported it appears that catalase of Johnson grass is rather sensitive to acids, but that it gives maximum activity in a considerable range of reaction from very slightly acid to rather markedly basic.

Table III shows the effect of the reaction of the dioxogen upon the catalase activity of a number of seeds. An examination of the table will show that unneutralized dioxogen inhibits in all and that the magnitude of the inhibition falls as the amount of powder increases. The plant material apparently acts as a buffer. In the after-ripened peach seed when 0.2 gm. of the seeds is used there is practically no inhibiting, while it is very marked with 0.05 gm. These relations for the peach are well shown by the curves in figure 1.

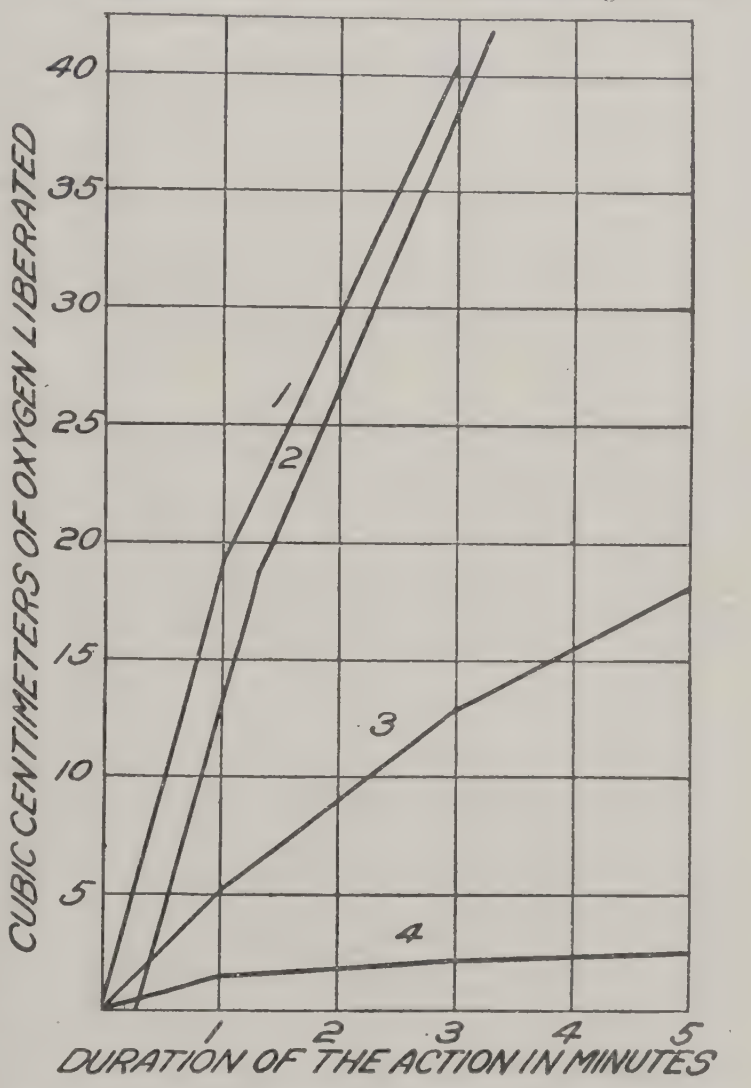

FIG. 1.—Graphs showing the effect of the acidity of dioxogen upon the catalase activity of after-ripened peach seeds; also the buffer effect of the seed material; 1, 0.2 gm. of seed material, dioxogen neutralized; 2, 0.2 gm. of seed material, dioxogen not neutralized; 3, 0.05 gm. of seed material, dioxogen neutralized; 4, 0.05 gm. of seed material, dioxogen not neutralized.

From these results it is evident that one must look after the reaction of the hydrogen peroxid in measuring the catalase of seeds. In his work with the potato tuber Appleman (1) maintained a neutral reaction by adding an excess of calcium carbonate. He showed that the emulsion of ground tuber either contained or developed sufficient acid to injure greatly the catalase after standing for a number of days and that this injury could be avoided by grinding the tuber with an excess of calcium carbonate. It is probable, however, that the low catalase activity of newly-ground tubers not treated with calcium carbonate was partly, if not mainly, due to the acidity of the hydrogen peroxid used.

DEGREE OF PULVERIZATION

The degree of pulverization has considerable effect upon the activity of catalase. As Table IV shows, Johnson grass gives maximum activity with medium fineness of the powder (70–80-mesh sieve) and very much less activity with fine grinding (200-mesh sieve), as well as with much coarser meal (40-mesh sieve). Crimson clover gives maximum activity

TABLE III.—*Effect of the reaction of the dioxogen upon the catalase activity of various seeds; also the buffer effect of the seed material*

[0.9 cc. of N/10 sodium hydroxid neutralizes 25 cc. of dioxogen to phenolphthalein. 100-mesh bolting cloth for clover; 70-mesh for other seeds.]

Seed used.	Powder used.	N/10 sodium hydroxid added per 25 cc. of dioxogen.	Oxygen liberated after—		
			1 min.	3 min.	5 min.
	Gm.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
Sudan grass	0.1	0.45	3.4	6.9	9.2
		.9	3.8	9.9	14.0
		.0	1.5	4.7	7.4
Amaranthus blitoides	.2	.9	6.1	11.8	14.7
		.0	4.8	9.0	10.9
Amaranthus retroflexus	.1	.9	4.9	9.0	11.1
		.0	2.4	4.5	5.6
	.2	.45	9.5	17.3	21.3
		.9	9.6	17.7	21.6
		2.7	9.0	16.7	20.2
		.0	6.7	12.5	15.0
Peach, dry-stored	.2	.9	.9	2.6	4.1
		.0	.9	2.5	4.0
Peach, after-ripened	.2	.9	18.7	40.5	.....
		.0	18.3	41.8	.....
Do	.05	2.7	5.7	15.7	21.6
		.9	5.2	12.8	18.1
		.0	1.5	2.2	2.5
Crimson clover (*Trifolium incarnatum*)	.05	.45	12.0	15.1	15.9
		.9	15.0	22.6	24.3
		2.7	15.8	23.8	25.9
		4.5	15.3	22.7	23.9
		.0	6.1	8.1	8.8

with 100-mesh and very nearly maximum with 200-mesh, but much reduced activity with 40-mesh. Agitation of enzyms generally causes degeneration. The fall in activity with finer pulverization may be due to the denaturing of the catalase by excessive mechanical manipulation in grinding and working the powder through the very fine bolting cloth.

TABLE IV.—*Effect of the degree of pulverizing upon catalase activity*

[Bolting cloth used: 70, 80, 100, and 200 mesh to the inch. The sieve used had circular holes 0.610 mm. in diameter]

Seed used and weight of powder.	Mesh of sieve per inch.	Oxygen liberated after—			
		1 min.	3 min.	5 min.	10 min.
		Cc.	*Cc.*	*Cc.*	*Cc.*
Johnson grass, 0.14 gm	200	3.1	6.8	9.2	13.9
	100	3.7	9.0	11.9	16.7
	80	4.9	10.5	14.4	20.3
	70	4.9	10.8	14.6	20.8
	(a)	3.1	7.3	10.5	15.2
Crimson clover, 0.07 gm	200	15.9	20.9	21.9	22.3
	100	16.1	22.1	23.6	24.7
	70	15.0	21.0	22.5	24.0
	(a)	7.5	12.1	14.5	17.4

[a] 0.610-mm. holes.

Table V shows the diameter of the cells of various parts of the two seeds mentioned in Table IV; also the pore diameter of some of the sieves used. Maximum activity is obtained when the average diameter of the sieve mesh is several times the diameter of the cells. The greater injury from fine pulverizing in Johnson grass may be due in part to the larger average cell diameter. Johnson grass catalase may also be relatively sensitive to mechanical agitation, as later sections show it to be to heat and aging effects. The endosperm of grasses has extremely low catalase activity, so it is left out of consideration in this connection. It is evident from the facts mentioned in this section that attention must be given to uniformity and fineness of pulverization.

TABLE V.—*Diameter of cells of various parts of seeds of Johnson grass and crimson clover*

Material.	Cell or mesh diameter.
Crimson clover:	μ
Cells of cotyledons	16– 40
Hypocotyl, except small cells of plerome	15– 30
Johnson grass:	
Scutellum, except elongated enzym layer	33– 66
Coleoptile and coleorhiza same, except small primordial cells	16– 33
200-mesh bolting cloth	33– 75
100-mesh bolting cloth	140–183
70-mesh bolting cloth	214–250
Sieve with 0.610-mm. holes	610

DEGENERATION OF THE POWDER

The powder ready for catalase determination degenerates rather rapidly when stored in a desiccator over quicklime. Table VI shows typical data for Johnson grass, the only species tested. This degeneration may be due in part to excessive drying, but it is probably in the main an aging change. A later section shows that there is a slow time degeneration in intact seeds. The morphological integrity, however, seems to secure very slow degeneration. From the results reported in this section it is evident that it is best to grind and screen the seed material just previous to making the catalase determinations.

TABLE VI.—*Degeneration of catalase in the powder of Johnson grass seeds*

Period of storage of powder.	Oxygen liberated after—	
	5 min.	10 min.
	Cc.	*Cc.*
1 day	13. 1	18. 6
54 days	4. 0	5. 8

In his work on plant oxidases Bunzell (*11*) frequently uses powdered plant material, but he has apparently made no statement as to the degeneration of the oxidase in it with storage.

VARIOUS ORGANS OF THE SEED

In the grains of grasses there is considerable difference in the catalase activity of different regions of the grain. Tables VII, VIII, IX, and X give data on this point. In Stoner wheat (Table VII) the catalase activity of the embryo is 28 to 29 times that of the endosperm. The complete caryopsis shows very low activity as compared with the embryo and less than twice that of the endosperm. The embryo of this wheat constitutes about 3.3 per cent of the caryopsis. The low catalase activity of the endosperm agrees with the claim of Grüss (*24*, p. *20–34*) and others that it is composed in the main of dead cells except for the aleurone layer. Burlakow (*4*) has found that the respiratory intensity of wheat embryos is about 20 times that of the endosperms. It seems then that in these seed organs high catalase activity parallels high respiratory intensity.

TABLE VII.—*Catalase activity of embryo, endosperm, and caryopsis of Stoner wheat; collected in August, 1917; run on October 5, 1917*

Part of seed used	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Caryopsis less embryo	0.8	1.4	1.7	2.0
Caryopsis	1.3	2.2	2.7	3.5
Embryo	23.3	39.3	.....	.....
Ratio, embryo to endosperm	29:1	28:1	.....	.....

With the smaller grass seeds it is an almost endless task to separate enough embryos to make catalase determinations, so in such grasses other methods were used for estimating the activity of various regions. In a fixed hybrid of Tunis grass and sorghum the catalase activity was measured for the caryopsis and the endosperm (Table VIII). The activity of the embryo was calculated from these data and from the fact that the embryo constitutes 9.6 per cent of the caryopsis. Such calculations tally closely with the actual measurements in the wheat, and it is probable they would do so here.

TABLE VIII.—*Catalase activity of the caryopsis and endosperm of a fixed hybrid of Tunis grass and sorghum seeds freshly harvested*

Region of seed used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Caryopsis less embryo	4.4	9.8	13.4	19.0
Caryopsis	11.3	22.2	29.2	37.9
Calculated for embryo	65.7	112.5	133.1	170.1
Ratio, embryo to endosperm	16.4:1	11.6:1	10:1	9:1

In Sudan grass the caryopsis was divided into a distal and proximal portion by cutting it crosswise just distal to the embryo. The proximal embryo portion was somewhat larger than the distal endosperm portion. As shown in Table IX, the embryo portion is more than twice as active as the complete caryopsis and several times as active as the endosperm end. Here, again, the embryo has very high catalase activity in comparison with the endosperm.

TABLE IX.—*Catalase activity of the distal and proximal ends of the caryopsis of Sudan grass collected at Khartum, Africa (1911?). Immature and mature grains separated with a vertical air-blast separator*

Sort of grain used.	Portion of caryopsis used.	Oxygen liberated after—			
		1 min.	3 min.	5 min.	10 min.
		Cc.	*Cc.*	*Cc.*	*Cc.*
Immature grains	Endosperm end	1.3	2.9	3.9	5.6
Do	Embryo end	17.5	32.0	38.5	.....
Do	Complete	6.4	12.5	16.3	22.2
Mature grains	Endosperm end	.8	1.8	2.4	3.5
Do	Embryo end	7.8	15.9	20.9	28.0
Do	Complete	3.8	7.5	10.1	14.2

Table X shows the relative activity of caryopses, the bracts inclosing them, and the sterile florets of Johnson grass. As one would expect, the nonliving and nonfunctioning parts show low catalase activity. They likely also show very low respiratory activity. The data of this section show that the catalase activity of the various organs of the grains of grasses parallels the physiological activity of these organs. The catalase in the endosperm, caryopsis bracts, and sterile florets may be a residuum of previous physiological activity. This seems to be the case, at least with the last two organs mentioned, for in them catalase activity shows an enormous fall with one year of dry storage. In this time it falls to one-seventh, or even one-tenth, its activity in the fresh but well-ripened seed. A later section shows a time fall in the catalase of the caryopsis of grasses, but the rate of fall in this is much slower.

TABLE X.—*Catalase activity of various organs in a sample of Johnson grass seeds*

Condition of plant.	Part of plant used.	Oxygen liberated after—	
		5 min.	10 min.
		Cc.	*Cc.*
1 year of storage	Caryopses	13.0	18.4
Do	Bracts surrounding caryopses	.5	.7
Do	Sterile florets	.6	.....
Freshly harvested but mature Johnson grass.	Caryopses	23.5	32.8
Do	Bracts covering caryopses	3.5	5.9
Do	Sterile florets	6.3	8.6

MATURITY OF THE GRAINS

The catalase activity of the grains of grasses is determined to a large degree by their maturity at the time of harvest. The immature grains have much higher activity than the mature ones. This holds for Sudan grass, as shown in Table VIII. This table also shows that the higher activity of immature grains is not lost with thorough drying, but that it is maintained after years of dry storage.

Since the immature caryopses are much smaller than the mature ones, the question naturally occurs, Is the activity per caryopsis about the same in mature and immature seeds? Table XI gives data that answers this question. In this experiment 10 seeds were used in each determination.

TABLE XI.—*Catalase activity of an equal number of mature and immature seeds of Sudan grass collected at Khartum, Africa (1911?)*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
10 immature, 0.05 gm.— of powder	2.1	4.0	5.3	7.2
10 mature, 0.07 gm.+ of powder	2.1	4.1	5.5	7.7

It is evident that the activity per caryopsis, whether mature or immature, is about equal. It is not known whether the embryo (the region of main activity) makes up a greater percentage of the caryopsis in the immature ones or whether there is about constant catalase activity per embryo regardless of size and maturity. The endosperms may also be more active in the immature caryopses.

Table XII shows the catalase activity of immature and mature caryopses of Johnson grass about two weeks after it had been harvested. The caryopses were removed from the bracts in a bunch of grains that ranged from medium to thorough maturity, and divided into two lots: Mature (large, plump, dark-brown caryopses), and immature (small, somewhat wrinkled, pink to light-brown caryopses).

TABLE XII.—*Catalase activity of mature and immature caryopses of Johnson grass; collected on September 14, 1917; run on September 28, 1917*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Immature	11.5	22.4	29.6	40.6
Mature	8.9	17.5	23.5	32.8

Newly harvested seeds of *Amaranthus retroflexus* show a similar relation between the catalase activity of immature and mature seeds. The data for this seed are given in Table XIII.

TABLE XIII.—*Catalase activity of mature and immature seeds of Amaranthus retroflexus; collected on September 14, 1917; run on September 24, 1917*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Mature	7.9	13.9	16.5	19.7
Immature	12.5	25.0	30.6	36.7

From the data of this section it is evident that in comparing the catalase activity of different lots of seeds one must be sure of the equal maturity of the lots compared. The experience of the writers shows that this can be approximated by the careful use of the vertical air-blast separator.

EFFECT OF DRYING ON CATALASE ACTIVITY OF SEEDS

In the case of peach and Johnson grass, drying the seeds after they have been in the germinator reduces the catalase activity very markedly in the first and noticeably in the second. Table XIV shows the reduction when slices of after-ripened peach seeds are dried rapidly before being ground. A considerably larger percentage of fall occurs in seeds that have been in the germinator for the same time at 20° or 25° C., although they have much lower absolute catalase activity, owing to their non-after-ripened condition. Intact seeds that have been dried for several days in the laboratory show a still greater percentage of fall. This also occurs where seeds are taken from the fresh fruit and allowed to dry.

TABLE XIV.—*Effect of drying seeds on catalase activity*

[Peach seeds (carpel removed) after-ripened at 7° C. for 54 days: one lot ground and used without drying, the other cut into thin slices and dried 3 hours before an electric fan previous to grinding. 0.1 gm. of material per run]

Sample used.	Oxygen liberated after—		
	1 min.	3 min.	5 min.
	Cc.	*Cc.*	*Cc.*
Ground wet	10.3	26.4	38.3
Ground after drying	3.0	9.1	14.5

Table XV shows the considerable fall in catalase activity of recently harvested Johnson grass caused by a short sojourn in a germinator, also the less considerable additional fall due to drying. It seems that the

catalase activity of these seeds can be greatly reduced by repeated subjection to germinative conditions followed by drying.

TABLE XV.—*Effect of drying on catalase activity of Johnson grass; collected on September 22, 1917; run on October 22, 1917*

Treatment of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Stored dry	6.3	13.7	19.0	27.4
In germinator 14 days at 20° C.	4.9	10.2	14.2	21.2
In germinator 14 days at 20° C., then dried 2 days	4.3	9.1	12.6	18.5

Table XVI shows the fall in catalase produced by drying a sample of Johnson grass that has low activity, due to a year's sojourn in a germinator, at 20° C. The absolute fall is low, but the percentage fall rather large.

TABLE XVI.—*Effect of drying on catalase activity of Johnson grass in germinator for 1 year at 20° C.*

Treatment of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Ground imbibed	1.5	2.3	2.7	3.4
Ground after drying 5 days	.8	1.6	2.2	3.1

In the after-ripened seed of basswood the catalase activity seems to rise with a few days' drying. This rise is only apparent, however, for the pulverized material from imbibed seeds contains more moisture when weighed than does the material from dried seeds, while the same weight was used for the determinations in the two cases. For the same reason the effect of drying, in both peach and Johnson grass, was much greater than the figures above indicate. It was not considered worth while, however, to go to the considerable trouble of correcting for the differences in moisture in the dried and undried material, for it would make a difference only in the magnitude and not in the direction of the results.

RELATION OF AGE AND VITALITY OF SEEDS TO CATALASE ACTIVITY

A number of workers (*5*, *6*, *7*, *8*, *35*) have shown that the oxidizing and digestive enzyms of old seed still persist after the seeds have completely lost their vitality, but it seems that these enzyms also gradually degenerate with age, although their complete degeneration follows much later than the complete loss of vitality.

Table XVII shows the relation of catalase activity to age and vitality in the seeds of Johnson grass, Sudan grass, and *Amaranthus retroflexus*.

TABLE XVII.—*Relation of catalase activity and age in dry-stored seeds*

[Only mature seeds used. Catalase determinations made between Sept. 26 and Oct. 5, 1917]

Kind and source of seeds.	Date of collection.	Oxygen liberated after—				Germination temperature.	Percentage of germination in—			
		1 min.	3 min.	5 min.	10 min.		3 da.	5 da.	7 da.	15 da.
		Cc.	*Cc.*	*Cc.*	*Cc.*	°*C.*				
Johnson grass:										
Arlington Farm, Va.	1917	6.3	13.7	19.0	27.4	25–40	.....	.....	.....	54
Do.	1916	4.3	8.6	11.4	16.0	25–40	.....	.....	.....	98
Fort Worth, Tex.	1911	3.2	6.5	8.5	11.6	25–40	.....	.....	.....	79
Sao Paulo, Brazil.	1908	1.6	3.1	4.2	6.0	25–40	.....	.....	.....	00
Bushnell Co., St. Louis, Mo.	1905	1.7	3.3	4.4	6.3	25–40	.....	.....	.....	19
Soudan grass:										
Sherman, Tex.	1916	4.2	9.1	12.4	17.3	25	.....	99	.....	.....
Khartum, Africa.	1911	3.8	7.5	10.1	14.2	25	.....	88	.....	.....
Khartum, original importation.	1908	2.0	4.2	5.8	8.2	25	.....	98	.....	.....
Amaranthus retroflexus:										
Pullman, Wash.	1917	8.7	18.1	22.7	26.8	40	99	100	100	.....
Arlington Farm, Va.	1917	8.0	13.9	16.7	19.8	40	30	91	100	.....
Chicago, Ill.	1917	8.3	16.8	20.7	24.9	40	37	100	100	.....
Do.	1915	8.4	16.5	20.9	24.8	40	94	100	100	.....
Do.	1914	7.9	15.7	19.2	22.3	40	100	100	100	.....
Allenton, Mo.	1894	8.5	13.8	15.5	18.5	40	00	00	00	.....
Fort Collins, Colo.	1894	6.3	12.5	15.2	17.0	40	00	00	00	.....
East Lansing, Mich.	1894	8.7	18.1	22.7	26.8	40	00	00	00	.....
Amaranthus graecizans:										
Pullman, Wash.	1917	4.4	8.5	10.5	13.0	40	12	85	100	.....
Amaranthus blitoides:										
Pullman, Wash.	1917	3.5	7.3	9.1	11.0	40	3	26	58	.....

In Johnson grass the fall evidently begins with harvest and continues for an indefinite time. The degeneration of catalase seems most rapid during the first year and slows down considerably during later periods. The same thing apparently happens in Sudan grass, but data are lacking for the 1917 crop. In both a very considerable fall in catalase activity occurs, with little or no fall in vitality. There is also considerable catalase activity when seeds fail to germinate at all. In the 9-year-old Sudan grass the percentage of germination is still very high (98 per cent), although the catalase activity has fallen to less than one-half that of the 1-year-old, and probably to less than one-third that of the freshly harvested. In the 9-year-old seeds there is somewhat lower vigor, as shown by the rate of growth of the seedling and the predisposition of the seed to fungus attack. In Sudan grass the catalase activity seems to be an excellent indicator of age. It is apparently a better indicator of age than it is of vitality. The same relation holds for Johnson grass, in which the catalase activity falls continuously with age except for the poor crop of 1908, which shows a slightly lower activity than the 1905 crop.

Figure 2 shows graphically the change in catalase activity and the percentage of germination with aging in Johnson grass seeds. The rather great irregularity in these curves is probably largely due to the diversity of source and handling of the several crops. In general, however, the catalase curve is concave upward while the vitality curve is convex. The rise in germination during the first year is due to after-ripening and not to increased viability, for treatment of the new seeds raised their germination to practically 100 per cent. It is interesting to note the similarity of these curves to the catalase viability curves in heated seeds given on p. 154. This similarity strengthens the coagulation conception of age degeneration (p. 154).

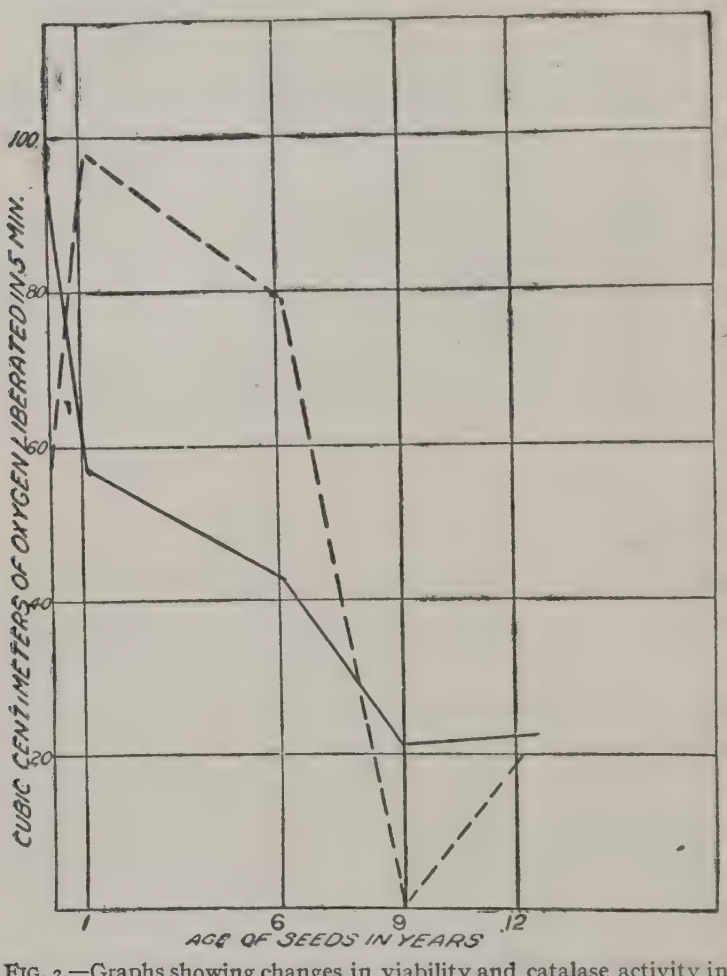

FIG. 2.—Graphs showing changes in viability and catalase activity in Johnson grass, caused by aging: Solid line=catalase; broken line=viability.

Catalase activity might easily be used as a method of estimating the age of seeds. If such were done, the following precaution would be necessary: The test must be compared with a crop of seeds of the same variety and of known age and like maturity, to serve as a standard. It could be applied only to seeds that have markedly time-labile catalase. It must be known that the seeds have gone through no drastic catalase-destroying condition, such as subjection to high temperatures or repeated wetting and drying. Before one could apply this method to any particular kind of seed he would have to know in detail the behavior of its catalase, and the method would show its main value when applied in conjunction with viability tests.

Practically all grass seeds go through a so-called after-ripening period following harvest. Air-dry storage or even storage in a drier condition seems to be a good, if not the best, condition for after-ripening. After-ripening is marked by improvement in rate and percentage of germination. The period of after-ripening is transient and is accompanied by only moderate improvement in germination in most of our readily ger-

minating grain grasses. In other grasses that are markedly resistant to germinating conditions the period is longer and the increase in germinative capacity very marked (*3*, *21*). Even in the grasses that ordinarily germinate readily, the dormancy may be deepened and the after-ripening rendered slow and important by certain conditions during ripening. This is true of "rain barley" (*36*) (barley ripening during rainy weather), and to a degree of "frosted grains" (grains frosted during the early stages of ripening) (*34*, *p. 436*). So far as studied, the dormancy in grass seeds seems to be imposed mainly by coat structures, and there is no evidence of rise in vigor of the embryo with after-ripening, as there is in peach and other seeds in which the embryos have a self-imposed dormancy. It is also interesting to note that in the grasses there is no rise but a very considerable fall in catalase activity with after-ripening, while, as shown in a later section, there is a great rise in catalase activity with after-ripening in seeds in which the embryos themselves determine the dormancy.

There is no evidence that the catalase of Johnson grass differs essentially either in amount or time lability from that of Sudan grass, although the seeds of the former are very refractory to germination conditions, and the latter respond readily.

In the seeds of *Amaranthus retroflexus* there is considerable variation in the catalase activity of the several crops studied, but there is no regular fall with age or even viability of the seeds. The catalase of this seed seems to be far more nearly time stable than is that of the grasses studied.

Crocker and Groves (*17*, *23*) have offered considerable evidence for the conception that age degeneration of seeds is due to a time-temperature denaturing of certain colloids (probably proteins) of the embryo. There also seems to be a time denaturing of the catalase of seeds, but it does not parallel the time denaturing of the materials essential to viability. The time lability of substances connected with viability may be compared with the time lability of catalase. The former are relatively time-stable in seeds of Johnson grass and Sudan grass (at least for the early period of storage), while the latter is relatively time-labile. In species of Amaranthus the former are relatively time-labile, while the latter is nearly time-stable.

Evidence given in other parts of the paper indicates that catalase activity is more closely correlated with respiration intensity than it is with viability; but the correlation with respiration is evidently not universal, for one can hardly conceive that seeds of *Amaranthus retroflexus* that have died from age still maintain full respiratory vigor.

The slower or lower percentage of germination shown in the 1917 Johnson grass and two lots of the 1917 *Amaranthus retroflexus* seeds is due to the unafter-ripened condition and not to low vitality. The seeds of *A. blitoides* and *A. graecizans* show a much lower catalase activity

than the seeds of *A. retroflexus*. They also show slower germination. It is not known whether the low catalase activity in these two species is due to a relatively low percentage of embryo material (the main seat of catalase) in the seeds, to some other specific difference in these seeds, or to lower vigor. The slow germination, however, may represent an unafter-ripened or dormant condition rather than low embryo vigor.

Seeds of *Amaranthus retroflexus* after-ripen during the first three or four months in dry storage. There is no evidence of embryo dormancy here. After-ripening is not marked by an increase in catalase activity. In this respect it resembles Johnson grass, but, unlike seeds of Johnson grass, there is no fall in catalase activity with age.

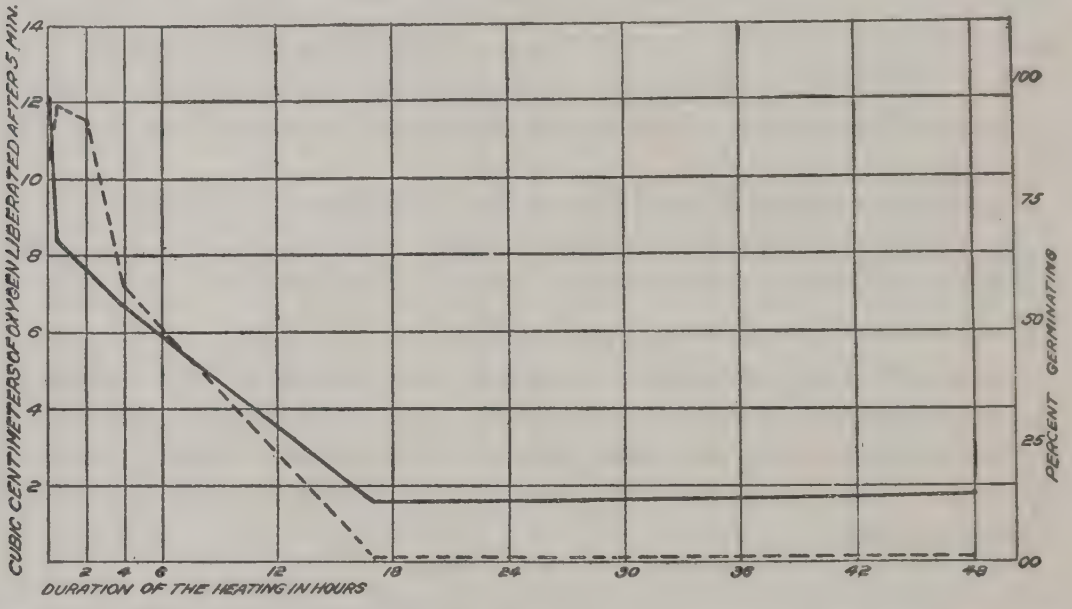

FIG. 3.—Graphs showing changes in viability and catalase activity in Johnson grass caused by heating air-dry seeds at 81° C. for various lengths of time: Solid line=catalase; broken line=viability.

EFFECT OF HEATING AIR-DRY SEEDS UPON THEIR VITALITY, CATALASE ACTIVITY, AND RESPIRATION

Heating air-dry seeds causes a fall in their vitality as well as in their catalase activity, but the denaturing of the substances connected with viability and of the catalase do not parallel each other (Table XVIII).

Heating Johnson grass to 81° C. for half an hour to two hours reduces the catalase activity by a large percentage and improves the germination. Longer heating (four hours at 81° C.) causes considerable additional reduction in the catalase activity and a very decided fall in germination. Still longer heating (17 hours, at 81° C.) reduces the catalase to from 10 to 16 per cent of its original value and kills all the seeds. Heating to 100° C. for five hours kills all the seeds and destroys all their catalase. In the early stages of heat degeneration, as in time degeneration, the catalase falls faster than the viability; but some catalase activity persists after the seeds are all killed. The relative rate of degeneration of catalase and vitality with heating is shown in figure 3.

In heated seeds of Johnson grass the catalase activity more nearly parallels the respiratory intensity than it does the viability or vigor of the seeds, for seeds heated at 81° C. for one hour show a great lowering in the respiratory intensity, but an increase in the rate of germination. Tables XIX and XX show this relation. The respiration rate was determined by the method described by Grafe (*22, p. 357–361*), with the exception that Reiset instead of Pettenkofer tubes were used for absorbing the carbon dioxid. The intensity of respiration is initially more than twice as high in the unheated as in the heated seeds. It rises in both with time, but heated seeds gain on the unheated. The gain in intensity in both is due to the initiation of germination and the faster gain in the heated seeds is caused by the more rapid germination in them.

TABLE XVIII.—*Effect of heating air-dry seeds upon their catalase activity and vitality*

Seed used.	Amount of heating.	Oxygen liberated after—				Percentage of germination in 9 days.
		1 min.	3 min.	5 min.	10 min.	
		Cc.	*Cc.*	*Cc.*	*Cc.*	
Johnson grass 1 year old.	None	4.4	9.3	12.7	17.8	82
Do	½ hour, 81° C.	3.1	6.2	8.4	11.6	95
Do	2 hours, 81° C.	2.9	5.7	7.7	10.3	92
Do	4 hours, 81° C.	2.6	5.2	6.7	8.4	49
Do	17 hours, 81° C.	.7	1.2	1.5	1.8	00
Do	48 hours, 81° C.	.6	1.4	1.7	2.2	00
Do	5 hours, 100° C.	0.0	0.0	0.0	0.0	00
Amaranthus retroflexus, 1917 crop.	None	7.5	12.5	14.5	16.9	100
Do	½ hour	.....	.....	.....	.....	25
Do	4 hours, 81° C.	5.2	9.3	10.9	12.4	00
Do	17 hours, 81° C.	4.7	7.1	9.3	10.7	00
Do	48 hours, 81° C.	4.6	7.8	9.1	10.3	00
Do	4.5 hours, 100° C.	.7	1.3	1.6	2.0	00

TABLE XIX.—*Effect of heating upon the respiratory intensity of 1-year-old Johnson grass seeds*

[Air-dry seeds heated 1 hour at 81° C., then soaked 24 hours at 20° C. Temperature of respiration chamber 20° C. Respiratory intensity=milligram of carbon dioxid produced by 10 gm., dry weight, of seeds per 24 hours]

Period.	Respiratory intensity.		Ratio, unheated to heated.
	Heated.	Unheated.	
First (17 hours)	4.5	9.6	2.1
Second (22 hours)	12.9	13.8	1.1
Third (18 hours)	20.2	17.8	.86

TABLE XX.—*Effect of heating 1 year old Johnson grass seeds for 1 hour at 81° C. upon rate of germination*

After various times at 25° C.	Percentage of germination	
	Heated	Unheated
28 hours	12	8
47 hours	43	18

It is evident that moderate heating of Johnson grass seeds reduces both their catalase activity and respiratory intensity, while it increases the germinative capacity. It seems to lead to a more economic use of food—that is, a lower percentage of it is respired and a larger percentage is available for building new organs. A number of substances are capable of modifying the economic coefficient of plants (*25*, *p.* 249–250). This may be part of the benefit claimed for heating seeds, while more rapid germination is also a part. It would be interesting to know how long this effect persists in the seedlings from heated seeds. The fact that the ratio is less than 1 in the third period (Table XIX) does not indicate that the effect is lost. This low ratio is probably due to the more advanced stages in the germination of the heated seeds, for Rischawi (*33*, *p.* 253) has shown that respiratory intensity increases as germination progresses in the grasses.

Table XVIII shows that seeds of *Amaranthus retroflexus* lose their viability rapidly, while the catalase falls relatively slowly with heating at 81° C. The vitality is reduced to 25 per cent after 0.5 hour and to nil after 4 hours of heating, while the catalase activity is still about 60 per cent of its original intensity even after 48 hours' heating at 81° C. In seeds of *A. retroflexus* the catalase is comparatively heat-stable, while the substances essential to viability are comparatively heat-labile.

The findings of the last two sections may be summarized by saying that the catalase in air-dry seeds of *Amaranthus* spp. is comparatively heat- and time-stable, while the substances connected with viability are comparatively heat- and time-labile. In the main, exactly the reverse is true for Johnson grass.

EFFECT OF RETENTION IN A GERMINATOR

The catalase activity of Johnson grass is greatly reduced by retention in a germinator. This effect is shown in Table XXI. The seeds in a germinator at 20° C. for one year show from one-third to one-sixth the catalase activity of those dry-stored for the same period. This fall in catalase activity evidently begins soon after the seeds are placed in the germinator, for the seeds in the germinator for one month at 20° C. have less than one-half the catalase activity of the same seeds dry-stored. The fall in catalase activity is also much slower at 7° C.

TABLE XXI.—*Effect of retention in a germinator on catalase activity of seeds of Johnson grass*

Treatment of seeds.	Oxygen liberated after—			
	1 minute.	3 minutes.	5 minutes.	10 minutes.
	C.c.	*C.c.*	*C.c.*	*C.c.*
Dry-stored 1 year (1916 crop)	4.8	10.1	14.3	21.1
In germinator 1 year at 20° C.	1.5	2.3	2.7	3.4
Dry-stored 1 month (1917 crop)	7.2	15.7	21.5	31.3
In germinator 1 month at 20° C.	3.5	6.7	8.8	12.3
In germinator 1 month at 7° C.	4.3	9.4	13.1	18.6

This fall in catalase is accompanied by a fall in respiratory intensity. Table XXII shows the rate of respiration of two samples of the same collection of Johnson grass seeds, one stored dry and the other kept in a germinator at 20° C. for one year.

The gradual rise in the respiratory intensity of dry-stored seeds is due to the initiation of germination. At the close of the third period several had germinated in this lot, while none had germinated in the dormant lot.

TABLE XXII.—*Effect of retention in a germinator on the respiratory intensity of Johnson grass seeds*

[Intensity in milligrams of carbon dioxid per 10 grams, dry weight, per 24 hours at 20° C.]

Treatment of seeds.	Respiratory intensity.		
	First period.	Second period.	Third period.
1 year in germinator at 20° C.	3.6	3.4	3.1
1 year, stored dry	9.5	10.6	12.9

When freshly harvested Johnson grass seeds are put into a germinator at 20° C., they become more dormant. The senior writer (*15, p. 110-117*) has called this "secondary dormancy" and has mentioned that unfavorable germination conditions produce this in many seeds. If this deepened dormancy is generally accompanied by lowered respiration, it may have an important bearing upon the duration of dormancy of some seeds in nature. It is conceivable that imbibed dormant seeds in the ground may finally exhaust their stored foods by respiration and thus set a limit upon their longevity. Leaching may also play a part.

Reduction of the respiration, as in Johnson grass, will lengthen the period necessary for exhausting stored foods. If 75 per cent of the weight of the seed can be respired before death occurs, secondarily dormant Johnson grass seeds could lie in a germinator for 9.8 years at 20° C. before death would occur from exhaustion of stored foods. The period at 10° C. would likely be 2 to 3 times 9.8 years, in accord with the temperature quotient for respiration (*26, p. 153–154*). Without such a reduction in

respiratory intensity the possible longevity would be a little more than one-third as great, figured on the initial rate in the active seeds. Even if the longevity of imbibed seeds in the soil be dependent upon some contingent other than exhaustion of stored food, this reduction in respiration is of significance. It will leave more stored material for building purposes in case germination does occur after a considerable period in the soil.

Atwood (*3*) observed that unafter-ripened seeds of *Avena fatua* become more dormant after they have been in a germinator for a few days. The power of the seeds to absorb oxygen falls with the deepened dormancy. This may be parallel to the reduced respiration just discussed for Johnson grass. There is also the possibility, however, that oxygen absorption in the wild oats is limited by permeability characters and not by respiratory capacity. There is need of following the changes in catalase activity as well as carbon-dioxid production during the acquiring of deeper dormancy in this seed as well as many others.

It is quite within the range of possibility that longevity of imbibed seeds in the soil is commonly limited by the exhaustion of stored foods by respiration. Seeds of *Amaranthus retroflexus* retain their viability in the soil for at least 30 years (*18*) and those of *Brassica nigra* for many years (*28*). Both absorb a considerable percentage of water. The same is probably true of many other seeds. In such seeds respiration must be at a very low intensity to avoid death from food exhaustion.

When the air-dry seeds (carpel removed) of the peach are placed in a germinator the catalase activity rises continuously for more than 30 days and probably for more than 54 days, as shown by Table XXIII. The rate of rise in catalase activity is very much greater at 7° C. than at 20° or 25°, and it is somewhat greater at 20° than at 25°. The temperature 5° has been shown to be very nearly the optimum for after-ripening of the embryo of *Crataegus* spp., and it is an excellent temperature, if not the optimum, for the after-ripening of the peach embryo as well as other dormant embryos. The temperature 7° proved very favorable for the after-ripening of the peach, as after 54 days many of the seeds showed signs of germination, and 10 days later all had germinated, while all those in the germinator at 20° and 25° were dormant, except for the small percentage that germinated the first few days, as always occurs when carpel-free seeds are put into a germinator at 20° or 25°. These produce only stunted seedlings.

It would be of interest to know whether rise in respiratory capacity accompanies the very marked rise in catalase activity during the after-ripening of peach seeds. Greatly increased vigor of the seedlings resulting from after-ripened seeds as well as the rather general parallel found between catalase activity and respiration would suggest increased respiratory capacity.

TABLE XXIII.—*Effect of the temperature of the germinator on the increase in catalase activity of peach seeds; collected on September 15, 1917, dried, free from carpels, and put into the germinator on October 11, 1917*

Treatment of seeds.	Period.	Oxygen liberated after—			
		1 min.	3 min.	5 min.	10 min.
	Days.	*C c.*	*C c.*	*C c.*	*C c.*
Dry-stored	9	1.0	3.1	4.7	9.7
In germinator at 20° C	9	3.4	9.6	13.2	20.4
In germinator at 7° C	9	4.8	11.9	16.9	23.6
Dry-stored	30	.9	2.8	4.2	.....
In germinator at 25° C	30	4.8	11.7	16.0	21.9
In germinator at 20° C	30	5.4	12.2	16.7	22.7
In germinator at 7° C	30	13.4	29.3	36.2	.....
Dry-stored	54	.9	2.6	4.1	.....
In germinator at 25° C	54	5.2	13.8	19.8	.....
In germinator at 20° C	54	5.1	15.3	23.0	.....
In germinator at 7° C	54	15.8	41.2	57.0	.....

Certain contrasts between the behavior of peach and Johnson grass seeds are evident and very important.

Air-dry seeds of peach have very low catalase activity when compared with air-dry seeds of Johnson grass, Sudan grass, and other seeds of the same year's collection. This difference is magnified when it is recalled that the peach material is all from the embryo, the active part of the seed, while that of Johnson grass is only about 10 per cent embryo with about 90 per cent endosperm, material of low activity.

The peach seeds rise in catalase activity when being kept in the germinator, and the rise is much faster and greater at 7° than at 20° or 25° C. Johnson grass seeds fall in catalase activity with retention in the germinator, and the fall is more rapid at 20° than at 7°.

After-ripening in the peach involves fundamental time-requiring changes in the embryo. It progresses rapidly in a germinator at a low temperature, apparently not at all in dry storage, and very slowly, if at all, in a germinator at 20° C. or above. It is marked by a very great rise in catalase activity. After-ripening in the Johnson grass does not seem to involve fundamental time-requiring changes in the embryo. It proceeds well, if not best, in dry storage and is accompanied by a fall in catalase activity.

Other seeds (hawthorn and basswood) with dormant embryos behave like the peach in after-ripening. As in the peach, the catalase changes are accompanied by other chemical changes, already mentioned in the introduction.

RISE OF CATALASE ACTIVITY WITH GERMINATION

There is a big rise in the catalase activity of Sudan grass seeds with their germination. This is well shown in Table XXIV for the 1916 crop of Sudan grass. In this experiment the seeds were used without separating them into mature and immature lots. In the germinated

lot the seeds were grown at 20° C. until the coleoptiles were 4 to 6 cm. long. The germinated seeds were then allowed to dry for seven days in laboratory air before they were ground. As is seen from Table XXIV, the catalase activity is about doubled by germination to the stage reported above. Germinated Johnson grass seeds showed similar behavior. The reported activity for germinated seeds may be low, for, as has been shown, drying commonly decreases catalase activity in seeds. There seems no doubt that this rise in catalase activity is accompanied by a rise in respiratory intensity, for Rischawi (*33, p. 253*), has shown that when wheat grains germinate and grow in darkness at 21° C. the respiratory intensity rises from a value of about 14 the first day to 50 on the tenth day, where it is maintained until the sixteenth day. It gradually falls from there on, owing to the exhaustion of stored foods, until it attains a value of 15 on the twenty-sixth day.

TABLE XXIV.—*Catalase activity of germinated and ungerminated Sudan grass seeds*

Condition of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Dry-stored	9.4	21.4	29.0	38.4
Germinated, coleoptile 4–6 cm	19.8	42.8	58.0	76.4

SOLUBILITY OF THE CATALASE OF SEED

Loew (*30*) found that the catalase of various plants consisted of two sorts: insoluble, or α-catalase, and soluble, or β-catalase. The relative proportion of these two constituents varied greatly in different plants as well as in different organs of the same plant. It was thought well to see whether there is any correlation between the relative time and heat stabilities of the catalases of amaranthus and Johnson grass seeds and the proportions of insoluble and soluble catalases in them. The data on this point are reported in Table XXV. In these experiments either 0.2 gm. of seed powder suspended in 10 cc. of water or the filtered extract of 0.2 gm. of seed powder in 10 cc. of water was used for each determination. Ten cc. of dioxogen were added to this, and the volume of oxygen liberated was measured. An excess of calcium carbonate ($CaCO_3$) was kept in contact with the materials at all stages of the process to protect against injury by acids.

Four different treatments were used for the materials of each sort of seeds: (1) Powder added to the water just before the determination and the whole suspension used in the determination; (2) powder suspended in the water and shaken for one hour at 25° C. and the whole suspension used in the run; (3) powder treated as the last but only the filtrate passing through a C. S. & S. 595 filter paper used in the deter-

mination; (4) same as the last but only the filtrate passing through a porous clay (fine Berkefeld) filter used in the run.

TABLE XXV.—*Solubility of catalases of amaranthus and Johnson grass seeds*

Seed and treatment.	Oxygen liberated after—	
	5 min.	10 min.
Amaranthus retroflexus:	*Cc.*	*Cc.*
Powder	19. 0	23. 6
Powder shaken with water 1 hour at 25° C	15. 5	19. 1
Filtered extract (C. S. & S. filter No. 595)	9. 0	11. 0
Filtered extract (Berkefeld)	8. 5	10. 1
Johnson grass:		
Powder	11. 0	16. 2
Powder shaken with water 1 hour at 25° C	10. 8	15. 9
Filtered extract (C. S. & S. filter 595)	3. 2	5. 1
Filtered extract (Berkefeld)	1. 5	2. 1

In amaranthus shaking the powder with water for one hour reduced the catalase noticeably. The catalase of this seed seems to be sensitive to such agitation. Filtering through either the filter paper or the Berkefeld filter reduced the activity somewhat more than 50 per cent, the latter showing slightly the greater reduction. In the Johnson grass shaking for one hour with water gave scarcely any reduction in activity, while filtering through the filter paper gave a reduction of about 70 per cent, and filtering through a Berkefeld filter a reduction of about 86 per cent.

On this basis somewhat more than 50 per cent of the catalase of amaranthus seeds is insoluble, while 70 per cent or more of that of Johnson grass seeds is insoluble. This, however, takes no account of the portion of catalase that may be chemically united with or adsorbed by the insoluble seed powder and the filters. It is likely also that the catalase complex is in the colloidal state and that the portion in the lower degrees of dispersion is held back by the fine filter, especially after it is blocked by colloidal materials. The proportion of soluble and insoluble catalase in these two seeds is not such as to throw any light on the relative time and heat stability of the catalases of them.

OXIDASE IN SEEDS

All oxidase determinations were made at 30° C. in the Bunzell simplified apparatus (*10*) either with or without caustic boats suspended from the manometer for absorption of carbon dioxid. All the material used was ground so that it would pass through bolting cloth with 70 or 80 meshes per inch. Seeds of Johnson grass, Sudan grass, or Tunis grass-sorghum hybrid, when used without removing the scales, were ground until about 85 per cent by weight pass through the bolting cloth, the remaining 15 per cent being discarded.

On account of its relative inactivity, the quantity of material used was necessarily large, usually 250 or 500 mgm. for each determination. The quantity of reagents used was uniform in all determinations, 10 mgm. of a solid reagent or 2 drops of para-cresol for each determination.

The different experiments were run for a length of time varying from 4½ to 24 hours.

OXIDASE ACTIVITY TOWARD DIFFERENT REAGENTS

Ten different lots of seeds, comprising Johnson grass, *Amaranthus retroflexus*, Sudan grass, wheat, and a fixed Tunis grass sorghum hybrid were tested for oxidase activity with pyrogallol, pyrocatechol, and para-cresol.[1] With very few exceptions pyrogallol gave the greatest activity, para-cresol next, and pyrocatechol least (*11*). Frequently the oxidase reaction toward pyrogallol and pyrocatechol proceeded at practically uniform rates throughout the experiment even when the experiment was run for nearly 24 hours. In some of the experiments, however, the rate slowed down perceptibly after the first few hours, although it never reached a definite end point with any of the reagents used. This is in marked contrast to the course of oxidase activity shown by Bunzell (*9*) with a large variety of plant materials. In his work the reaction seemed to be practically complete in 2 or 3 hours.

The activity towards para-cresol usually started very slowly (probably on account of the limited solubility of the reagent), increased somewhat in rate after the first few hours so that the total reduction in pressure sometimes temporarily exceeded the total reduction with pyrogallol as the reagent, and then decreased in rate so that the pressure reduction again became considerably less than with pyrogallol.

All of the material used had relatively low activity. The greatest activity of the most active material used (except scales and sterile florets) caused a total reduction of pressure of about 95 mm. in 19 hours with 250 mgm. of the material and 17 cc. as the active volume of gas. This is equivalent to the absorption of only about 10.7 cc., or 15 mgm. of oxygen in 24 hours per gram of the ground seed material; yet this is more than 10 times as great an activity as that shown by ground peach embryos. Johnson grass seeds were more active than any other seeds used, with amaranthus a close second. The Tunis grass-sorghum hybrid was somewhat less active, and Sudan grass (1911 seed) very much less active. Wheat (entire caryopses) showed very little oxidase activity, and peach embryos practically none at all.

DETAILED ACCOUNT OF OXIDASE EXPERIMENTS

The results given in the following paragraphs were obtained with pyrogallol as the reagent. The amount of oxidase material used was not

[1] Preliminary trials with Johnson grass seeds showed practically no activity toward hydroquinone and tyrosine.

uniform in the different experiments. When different amounts of the same material were used in duplicate determinations the smaller quantity invariably showed greater relative activity than the larger quantity. On account of the lack of proportionality between the amount of seed material used and intensity of oxidase activity indicated, the latter is reported in the following paragraphs in terms of reduction of pressure in a given time, with a given amount of seed material.

OXIDASE ACTIVITY IN EMBRYO AND ENDOSPERM

The material used was Stoner wheat, harvested in July, 1917, and well-matured Sudan grass seed about 6 years old, the entire caryopses ground whole and the endosperm and embryo ends ground separately being used. For this purpose the Sudan grass caryopses were simply cut in two just distal to the embryos. The wheat kernels were cut diagonally so as to include only a small amount of endosperm with the embryo. The embryo ends thus cut off constituted 11.3 per cent of the entire caryopses. The embryo ends were ground until 8.7 per cent by weight of the entire caryopses passed through the bolting cloth, the residue of endosperm and bran being discarded. The remaining 88.7 per cent of the caryopses was ground until 34.7 per cent of the entire caryopses, consisting of the inner portion of the endosperm, passed through the bolting cloth; the remainder was discarded.

Table XXVI shows the oxidase activity of endosperm and embryo ends compared with that of the entire caryopses. The embryo ends showed very much greater activity than the endosperm ends, while the activity of the whole caryopses was intermediate.

TABLE XXVI.—*Oxidase activity of embryo and endosperm ends of caryopses compared with that of the entire caryopses*

Material.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, embryo to endosperm.
Stoner wheat:	*Mgm.*		
Whole caryopses	500	9.5 mm. in 4½ hours	
		55.5 mm. in 19½ hours	
Embryo ends [a]	500	27 mm. in 5 hours	54
Do	250	17.5 mm. in 5 hours	
Endosperm ends [b]	500	0.5 mm. in 5 hours	
Sudan grass seed (1911?):			
Whole caryopses	400	15.5 mm. in 7 hours	
Embryo ends	500	22 mm. in 6 hours	5.5
Endosperm ends	500	4 mm. in 6 hours	

[a] 8.7 per cent, by weight, of the grain, ground fron 11.3 per cent portions were used. The coarse residue was discarded.

[b] 34.7 per cent, by weight, of the grain, ground from 88.7 per cent portions were used. The coarse residue was discarded.

OXIDASE ACTIVITY AS RELATED TO MATURITY OF SEED

The material used was Sudan grass seed about 6 years old and Johnson grass seed about one month after harvesting. The separation of the

large, heavy, thoroughly mature seeds from the lighter, more poorly matured seeds was made by means of vertical air-blast seed separator. The results of the experiment are given in Table XXVII.

TABLE XXVII.—*Oxidase activity of mature and immature seeds*

Material and condition.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, immature to mature.
	Mgm.		
Sudan grass, whole caryopses:			
Mature	400	15.5 mm. in 7 hours	0.84
Immature	400	13 mm. in 7 hours	
Sudan grass, embryo end:			
Mature	500	22 mm. in 6 hours	
Immature	400	15.5 mm. in 6 hours	
Johnson grass, caryopses in scales:			
Mature	500	35 mm. in 6 hours	1.30
Do	500	45.5 mm. in 6 hours	
Immature	400	42 mm. in 6 hours	

The mature Sudan grass seed was slightly more active than the immature, whether the whole caryopses or only the embryo ends were used. With Johnson grass seed, however, the immature seed was considerably more active than the mature seed.

OXIDASE ACTIVITY AS RELATED TO AGE OF SEED

The material consisted of a sample of Johnson grass seed about 3 years old; two samples of Johnson grass seed from a common original selection, one of which was 1 year old and the other about 1 month old; and two samples of seed of *Amaranthus retroflexus*, one over 2 years old and the other about 2 weeks after harvesting. The results of the experiments are given in Table XXVIII.

TABLE XXVIII.—*Oxidase activity as related to the age of the seed*

Material.	Age.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, old to new.
		Mgm.		
Johnson grass 1417	3 years	500	10.5 mm. in 4½ hours	0.35
Johnson grass 8599	1 year	500	29.5 mm. in 4½ hours	
Do	do	250	68 mm. in 19 hours	.72
Do	3 weeks	250	95 mm. in 19 hours	
Amaranthus retroflexus	2 years	500	19.5 mm. in 4½ hours	.51
Do	2 weeks	500	38.5 mm. in 4½ hours	
Do	2 years	250	25.5 mm. in 10 hours	.57
Do	2 weeks	250	45 mm. in 10 hours	

The intensity of oxidase activity decreases markedly with age. One-year-old Johnson grass seed was a little more than two-thirds as active

as fresh seed; two-year-old amaranthus seed was about one-half as active as fresh seed; and three-year-old Johnson grass seed was only about one-third as active as one-year-old seed.

OXIDASE ACTIVITY AS RELATED TO AFTER-RIPENING AND CONDITION OF STORAGE OF SEED

The oxidase activity of samples of Johnson grass seed a little over a year old, which had been stored dry in cloth sacks at room temperature, was compared with that of portions of the same original samples which had been stored for a year between moist blotting papers at 20° C.[1] Comparative tests were made also with peach embryos which had been stored for 1 month air-dry at room temperature and in moist blotting paper at 7° C.,[2] and similarly at 25°. The peach seeds were taken from the stony carpels before the period of storage, and the coats were removed from the embryos just before grinding the latter for oxidase determinations.

Table XXIX gives the results of the experiments. One-year-old Johnson grass seed which had been kept at a temperature slightly below the minimum for germination, though otherwise under germination conditions, showed considerably less oxidase activity than seeds from the same original lot which had been stored dry. After grinding both lots were dried in a desiccator before weighing. Peach embryos showed very little oxidase activity under any conditions, and this activity did not change significantly during incubation even at the after-ripening temperature, 7° C.

TABLE XXIX.—*Oxidase activity as related to after-ripening and condition of storage of seed*

Material and condition.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, wet to dry.
Johnson grass 8599 (collected on September 9, 1916):	*Mgm.*		
Stored dry 1 year at room temperature.	500	29.5 mm. in 4½ hours...	.....
Stored in germinator 1 year at 20° C.	250	13.5 mm. in 4½ hours...	.....
Johnson grass 8599 (collected on September 23, 1916):			
Stored dry 1 year at room temperature.	250	24.5 mm. in 6½ hours...	0.80
Stored in germinator 1 year at 20°C.	250	19.5 mm. in 6½ hours...	
Peach embryos:			
Stored dry at room temperature...	500	6.5 mm. in 24 hours.....	.....
Incubated 45 days at 7° C.....	500	3.5 mm. in 24 hours.....	.....
Incubated 45 days at 25° C.....	500	3.5 mm. in 24 hours.....	.....
Stored dry at room temperature...	250	9 mm. in 1½ 9 hours ...	.....
Incubated 42 days at 7° C.....	250	9 mm. in 1½ 9 hours ...	.....

[1] Johnson grass seeds were kept in a condition of secondary dormancy by storing in moist blotters at 20°.

[2] Peach embryos stored thus after-ripen rapidly at 7°, but not at 25°.

Supplementary experiments with peach embryos, with pyrocatechol and para-cresol as oxidase reagents, showed practically no oxidase activity either with dry-stored embryos or with after-ripened embryos. In this connection the color reaction in the oxidase reagents is of interest. The pyrocatechol and pyrogallol solutions became very slightly colored during the experiments. This coloring was barely perceptible with the material which had not after-ripened and slightly more intense with after-ripened material. With para-cresol as the reagent and in control lots with no reagent there was no change in color.

OXIDASE ACTIVITY AS RELATED TO GERMINATION

Johnson grass seeds collected on September 9, 1916, were incubated at 20° C. for several days late in November, 1917, and then with the temperature alternation 25° to 40° until nearly all had germinated. Seeds which showed no sign of germination were then picked out, ground, and tested for oxidase activity in comparison with dry seeds and with seeds which had germinated, and in comparison also with the sprouts produced by another sample of the same original lot which had been germinated at 20° after first heating for 1 hour at 81° and sterilizing with 5 per cent silver nitrate.[1]

Many of the seeds in the last-mentioned sample had reached an advanced stage in germination, some coleoptiles being over 10 cm. long. The elongated coleoptiles and a few of the roots were broken off, crushed, dried before a fan, and ground for oxidase determinations.

The germinated seeds which had been ground entire were in various stages of growth. The longest coleoptile was 8 cm. long and the longest root 4 cm., but a large majority were less than one-half as long as these. Before being ground these germinated seeds had been crushed and dried before a fan. Table XXX gives the results of these experiments.

TABLE XXX.—*Relation of oxidase activity to germination*

Material and condition.	Quantity of powder used.	Reduction of pressure.
Johnson grass 8604 (collected on Sept. 9, 1916):	*Mgm.*	
Ground dry	333	20.5 mm. in 7½ hours.
Incubated several days at 20° C., then at 25° to 40° C.—		
Not germinated	333	16.5 mm. in 7½ hours.
Germinated	333	16.0 mm. in 7½ hours.
Sprouts	167	14.0 mm. in 7½ hours.

[1] Although the sterilized seeds were very thoroughly washed, first with distilled water, then with sodium chlorid solution, and finally with distilled water, oxidase activity in the ground seeds was almost completely inhibited, probably by adsorbed silver. Of course the sprouts were free from this inhibiting agent.

The dry seeds apparently showed somewhat greater oxidase activity than the imbibed seeds, either germinated or not germinated. The differences, however, are probably only apparent, as the ground imbibed seeds at the time of weighing contained a higher percentage of moisture than the ground dry seeds. If one assumed a moisture content of 8 per cent for the powder from the dry seeds and a moisture content of 26 per cent for the powder from the imbibed seeds, the intensity of activity would be identical when calculated to a dry-weight basis.

The ground sprouts were considerably more active than the whole seeds, but the ratio is no greater than would be expected from the comparison of embryo ends with caryopses (see Table XXVI); therefore no increase in activity upon germination is indicated.

OXIDASE ACTIVITY OF NONLIVING STRUCTURES

A very interesting fact is the relatively high oxidase activity of nonliving structures in which catalase activity is very slight and respiration presumably absent—viz, the bracts or scales which inclose the mature caryopses of Sudan grass and Johnson grass and the dry abortive or sterile florets of Johnson grass.[1] Johnson grass and amaranthus seeds showed greater activity than the other kinds of seeds. It was at first thought that this fact might be related to the intense pigmentation of the scales in one case and of the pericarps in the other. As Table XXXI shows, comparative experiments with scales and caryopses proved the contrary.

TABLE XXXI.—*Oxidase activity of nonliving structures*

Material and condition.	Quantity of powder used.	Reduction of pressure.
Johnson grass 8599 (collected on Sept. 22, 1917), medium ripe:	*Mgm.*	
Caryopses	500	26 mm. in 5 hours.
Scales (medium colored)	250	24 mm. in 5 hours.
Johnson grass 8599 (collected on Sept. 9, 1916), very ripe:		
Caryopses (removed from scales by grinding in coffee mill, fall of 1916).	500	15 mm. in 5 hours.
Scales (black)	500	Do.
Sterile florets	400	20. 5 mm. in 1 hour. 31. 0 mm. in 2 hours. 49. 0 mm. in 5 hours.
Sudan grass, mature:		
Endosperm ends of caryopses	500	4 mm. in 6 hours.
Embryo ends of caryopses	500	7. 5 mm. in $2\frac{1}{6}$ hours. 22. 0 mm. in 6 hours.
Scales, light straw-colored	500	15. 5 mm. in 1 hour. 21. 5 mm. in $2\frac{1}{6}$ hours. 33. 0 mm. in 6 hours.
Scales, control, no reagent	500	6 mm. in $2\frac{1}{6}$ hours. 10 mm. in 6 hours.

[1] Each fertile floret in Johnson grass is accompanied by a sterile floret which never develops a caryopsis.

The scales of only moderately well matured Johnson grass seed showed a somewhat greater oxidase activity than the caryopses, but with unusually well matured 1-year-old seed having intensely pigmented scales the activity of the scales was exactly the same as that of the caryopses. At the same time the sterile florets, though functionless structures scarcely pigmented at all, in which all vital activities must have ceased at a time corresponding to an early stage in the development of the caryopses in the accompanying fertile florets, were about four times as active as either scales or caryopses. In fact, these sterile florets showed greater oxidase activity in limited time (5 hours) than any other material studied in the investigation.

The light, straw-colored scales of Sudan grass showed 50 per cent greater total oxidase activity in 6 hours than the embryo ends of the caryopses, and about 8 times as great as the endosperm ends.

A very noticeable feature of the high oxidase activity of Sudan grass scales and sterile florets of Johnson grass is the high initial rate of activity followed by a rather rapid and progressive decline in rate, though activity had not ceased when the experiments were concluded. As shown in Table XXXI, the decrease in pressure with this material was nearly half as great at the end of 1 hour as at the end of 5 or 6 hours. This is in marked contrast to the progress of the reaction with the other kinds of material used in the investigation, and suggests rather the type of reaction reported by Bunzell. By the end of the second hour the initially high rate of activity of Sudan grass scales fell below the constant rate maintained by the embryo ends of the caryopses.

It is interesting to notice that there was a similar reaction with decrease in rate after the second hour in a control with ground scales and no reagent. In this control tube the reduction of pressure was 6 mm. in $2\frac{1}{6}$ hours, and 10 mm. in 6 hours. With most of the other kinds of material used, the controls showed little or no change in pressure until fermentation began, after which the reduction in pressure was frequently rapid. No control was run with the sterile florets.

One might perhaps see a correlation between the high oxidase activity of these structures (scales and sterile florets) and the high oxidase activities caused by agencies which retard normal growth, as reported by Bunzell (*9*). He suggests a general relation between retardation of growth, from any cause whatever, and rise in oxidase activity. In the case of the sterile florets retardation or suspension of function has proceeded so far as entirely to prevent the formation of seeds and to withdraw all vitality from the florets.

EFFECT OF MERCURIC CHLORID UPON OXIDASE ACTIVITY

Previous work has shown that mercuric chlorid ($HgCl_2$) has a strong forcing action upon the caryopses of Johnson grass, the maximum effect being obtained with a *M/2,000* solution. Table XXXII shows the

oxidase activity of 250-mgm. samples of fresh seed of Tunis grass-sorghum hybrid and of Johnson grass seed in secondary dormancy with *M/2,000* mercuric chlorid replacing distilled water in the oxidase baskets. The results with distilled water are also given.

The mercuric-chlorid solution had only a slight depressing effect upon oxidase activity. Either this oxidase is very much more resistant to mercury poisoning than some other enzyms or else the concentration of the solution was very greatly reduced by adsorption to the large amount of powder used. McGuigan (*31*) found the activity of diastase completely inhibited by *M/30,000* mercuric chlorid. Caldwell (*14*) showed that the activity of bromelin was completely inhibited by *M/45,000* to *M/75,000* mercurous nitrate ($HgNO_3$) and mercuric nitrate $Hg(NO_3)_2$.

TABLE XXXII.—*Effect of M/2,000 mercuric chlorid upon oxidase activity*

Material.	Reagent.	Reduction of pressure.
Tunis grass-sorghum hybrid (collected on Sept. 14, 1917.)	Pyrogallol + *M/2,000* mercuric chlorid.	16 mm. in 6 hours.
Do	Pyrogallol in distilled water.	20 mm. in 6 hours.
Johnson grass 8599 (collected on Sept. 23, 1916), incubated at 20° C.	Pyrogallol + *M/2,000* mercuric chlorid.	12.5 mm. in 6 hours.
Do	Pyrogallol in distilled water.	14 mm. in 6 hours.

SUMMARY

(1) Measurement of the oxygen liberated from hydrogen peroxid by the addition of an excess of powdered seeds (plant catalase) provides a convenient method of determining the concentration of the hydrogen peroxid used.

(2) In the determination of the catalase of seeds it is necessary to neutralize the hydrogen peroxids used, for the acidity in all commercial hydrogen peroxids tried was sufficient to reduce greatly the catalase activity. The seeds studied bear no inhibiting acids, but show buffer action against the acids of the hydrogen peroxids.

(3) Excessive pulverization of seeds reduces their catalase activity. Powder of Johnson grass gave maximum activity when passed through a 70-to-80-mesh bolting cloth, and crimson clover when passed through a 100-mesh.

(4) In powdered seed material (Johnson grass) stored in a desiccator at room temperature the catalase degenerates rather rapidly, losing 70 per cent of its activity in 54 days. Morphological integrity insures much slower degeneration.

(5) In the embryo of wheat the catalase activity is 28 to 29 times that of the endosperm. Burlakow (*4*) found the respiratory activity of the

embryo 20 times that of the endosperm. In other grasses studied the catalase activity of the embryo was many times that of the endosperm. The oxidase activity is likewise much higher in the embryo than in the endosperm.

(6) The physiologically inactive organs (sterile florets and caryopsis scales) of grass seeds show only a small fraction of the catalase activity shown by the caryopses. This likely agrees with the respiratory intensity. The oxidase is as active, or in some cases several times as active, in the nonliving as in the living organs.

(7) Equal weights of immature caryopses of Johnson grass or Sudan grass and of seeds of *Amaranthus retroflexus* give much greater catalase activity than mature ones. The activity of an equal number of the caryopses, mature and immature, of the two grasses is about equal. The oxidase activity on weight basis in Sudan grass is slightly higher in mature seeds than in immature, and the reverse holds for Johnson grass.

(8) Drying the seeds that have been in a germinator reduces enormously the catalase activity in the peach, noticeably reduces it in Johnson grass, but not at all in basswood.

(9) The catalase in air-dry Johnson grass seeds is comparatively time- and heat-labile, while that in air-dry amaranthus seeds is relatively time- and heat- stable. The respiratory intensity (measured after the seeds are imbibed) parallels the catalase activity in the first species. There is nothing to indicate such a relation in the latter. The difference in time and heat stability is not determined by the relative proportions of soluble and insoluble catalases in the two seeds. The nonliving organs of Johnson grass (sterile florets and caryopses scales) show much faster time degeneration of their catalase than the living embryo.

(10) Retention in a germinator, not furnishing conditions for germination, greatly reduces the catalase activity of Johnson grass seeds. One year at 20° C. reduces it more than 66 per cent, and 1 month more than 50 per cent below that of the same crop dry stored. The rate of fall in the catalase activity is decreased by lowering the temperature of the bath. Retention in a germinator affected the oxidase activity in the same direction but to a much less degree.

(11) The fall in catalase activity mentioned in the preceding paragraph is accompanied by a commensurate fall in respiratory intensity. A similar response seems to occur in *Avena fatua*, and probably occurs in many other seeds. This reduction in respiratory intensity is of great significance in conserving stored foods in seeds lying in the ground, dormant and imbibed, for many years.

(12) The catalase activity in dry peach seeds is very low, but rises as the seeds lie in the germinator imbibed. The rise continues for weeks and is much more rapid at 7° C. than at 20° or 25°. The optimum temperature for after-ripening seems to be optimum for catalase increase. In other seeds having dormant embryos, so far as studied by other investi-

gators, the same relation holds. In the peach the oxidase activity (Bunzell method) decreases with after-ripening but autocoloration of the ground seed mass exposed to the air increases.

(13) In amount of catalase and in the general behavior of their catalases Johnson grass and Sudan grass seeds are very similar and one finds here no explanation for their marked difference in dormancy and requirement of alternate temperatures for germination. The same is true of the oxidases of the two seeds, so far as our studies have gone.

(14) The catalase activity of grass seeds rises rapidly as their germination progresses. This parallels the rise in respiratory intensity. There is no rise in oxidase activity with germination.

(15) In Johnson grass seeds there seems to be a close correlation between catalase activity and respiratory intensity (factors that modify one modify the other similarly), but there is not a very close correlation between either of them and the vitality of the seeds or vigor of the resulting seedling. In these seeds catalase determination proved an excellent quick method of estimating respiratory intensity and led to the discovery of several interesting features in their respiration. In these seeds the catalase also decreases with age and it is a fair measure of age in continuously dry-stored seeds.

(16) In amaranthus seeds there is no evidence of a correlation between catalase activity on one hand and respiratory intensity, vitality, or age on the other. This lack of correlation may be connected with the greater time and heat stability of the catalase of amaranthus.

(17) So far as studied to date, seeds that after-ripen with dry storage but which do not have embryos with dormancy self-imposed at any time either show no change in the catalase activity (amaranthus) or a decrease in it (Johnson grass) with after-ripening.

(18) Seeds that after-ripen in a germinator at low temperature (commercial layering) and in which the dormancy of the embryo is self-imposed and the embryo experiences fundamental time-requiring changes for after-ripening, show a great increase in catalase activity with after-ripening (hawthorns, basswood, peach).

(19) It has been suggested that the gradual loss of vitality in dry-stored seeds with age is due to the time denaturing or time coagulation of embryo proteins. If this be true, it is evident that the time denaturing of the embryo proteins essential to vitality and the time denaturing of catalase are quite distinct, for in no old seeds studied is there a close parallel between catalase activity and vitality.

(20) It is evident from the great variations in catalase behavior in the several seeds studied that one can not draw general conclusions for the catalase behavior in all seeds, but it seems evident from the data in this paper that seeds will fall into several physiological types for each of which more or less general conclusions can be drawn.

(21) Catalase activity of seeds seems to parallel physiological behavior much more generally than does oxidase activity.

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THE MEADOW PLANT BUG, MIRIS DOLABRATUS[1]

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INTRODUCTION

The meadow plant bug, *Miris dolabratus* L., presents a number of interesting problems, biologic as well as economic, and, considering its great abundance over a large area of the eastern United States and Canada during the past 40 years, it seems strange that it has not received more careful investigation.

My own attention was attracted by its appearance in immense numbers in northern Ohio at about the time of my removal to that State in 1898. It was entirely unknown to me from previous field collecting, and specimens I had seen had been collected in western New York by Mr. E. P. Van Duzee about the year 1888.

My attention was again forcibly attracted to the species by its great abundance in Maine in 1914, when I encountered it while studying the meadow leafhoppers. Reference to the literature indicated the almost total neglect of the species in this country, and almost nothing concerning its economic importance was found. It seemed, therefore, well worth a special study, and I was gratified to be able to arrange with the Maine Agricultural Experiment Station to undertake a summer's study of the species at Orono.

As an Old World species the insect has evidently been familiar since it was described by Linnæus (*1758, p. 449*),[3] and has had frequent mention by later writers, who have treated it simply from the systematic standpoint. Wolff (*1802, p. 115–116, fig. 109–110*) indeed gives a recognizable figure of the nymph in one of the later instars, also a rough sketch of the egg; but, so far as noted, no detailed study of the life history, habits, or economic status has been made, even in the regions where it has been longest known.

DISTRIBUTION OF THE SPECIES

The range of the species is evidently throughout the Palearctic region, as the European records cover the territory to the Mediterranean, and the Asiatic seem to include all north of the Himalaya Mountains at least.

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[1] Papers from the Maine Agricultural Experiment Station: Entomology 99. Contribution from the Department of Zoology and Entomology, Ohio State University, No. 53.

[2] I am indebted to a number of persons for assistance in the preparation of this report, especially to Dr. Edith M. Patch, of the Maine Experiment Station, for facilities to carry forward the study and to Mr. R. K. Fletcher for careful attention to field observations and to the entomologists of various States who have kindly taken the trouble to send records for their territory.

[3] Bibliographic citations in parentheses refer to "Literature cited," p. 199–200.

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Oshanin (*1909, p. 779*) lists the distribution as—

Scandinavia, Batavia, Britannia, Belgica, Germania, Helvetia, Gallia, Lusitania, Hispania, Moldavia, Serbia, Romania, Hungaria, Rossia fere tota, Caucasus, Sibiria. Regio nearctica (Canada, eastern United States).

PUBLISHED RECORDS OF OCCURRENCE IN AMERICA

The first record of the species in America that I can identify as referring to its occurrence in America is the one by Uhler (*1878, p. 597*). Provancher (*1872, p. 78*) recorded it for the vicinity of Quebec when he listed it as a new species under the name "*Miris belangeri*" in 1872 and later (*1886, p. 104*) referring it to the European species under the name "*Leptoterna dolabrata* L." In Uhler's Check List of Hemiptera Heteroptera (*1886, p. 17*) it appears under the name "*Leptoterna dolobrata*" with locality as "E. St." Van Duzee (*1887, p. 70*) says:

May to August. In dry fields. Probably our most abundant Hemipter. It attains full development about June 1, and frequently appears in immense swarms in favorable localities.

Later (*1894, p. 176*) he says:

Often appears in immense swarms toward the last of June on grass in hayfields and pastures.

Van Duzee (*1905, p. 550*) also records the species for the Adirondack Mountains and (*1908, p. 111*) for Quinze Lake, Province of Quebec, Canada, in 1907. Slosson (*1894, p. 5*) records the species for Mount Washington, New Hampshire, above the 5,500-foot altitude.

Webster and Mally (*1897, p. 41*) barely mention the species as abundant on the heads of timothy in 1896, which is the first published record for Ohio, though Mr. Hart says a specimen is in the Illinois collection sent from Columbus by Prof. C. M. Weed, presumably about 1888 or 1890.

The species is recorded for New Jersey for a number of localities by Smith (*1900, p. 128; 1910*), but with no definite dates. It is also listed by me (*1900, p. 70*) in the Ohio list, and its abundance in Maine is referred to by Patch (*1908, p. 303*) and by me (*1916, p. 50*).

Finally a record of the rearing of a parasite, *Phorantha occidentis*, by Leonard (*1916*) indicates its occurrence in 1915 in New York.

None of these records, except Uhler's, raises the question of the derivation of the species, but from the facts that there were no early records of damage in this country and that there seemed to be a distinctly westward dispersal the possibility of its being an introduced species associated with timothy (*Phleum pratense*) as its principal food plant seemed to warrant an effort to determine this point.

The following letter with note concerning the species was sent to a number of entomologists in the various States and to Dr. C. Gordon Hewitt, Dominion Entomologist, of Canada.

ORONO, MAINE, *July 5, 1916.*

DEAR SIR: The writer is engaged upon a study of a meadow Capsid (*Miris dolabratus*) for the Maine Agricultural Experiment Station, and since the species is abundant over a considerable area of the northeastern United States it is desirable to secure data from as large an area as possible. With the cordial approval of the Station authorities and in the hope that the results may have more than local value I am asking assistance in securing such data from the entomologists in a number of adjacent states. The data desired covers such items as occurrence, abundance, recognized injury, grasses affected and any matter bearing on the life history and habits in the localities reported. Any such information will be welcomed and duly credited.

The species is one of the larger Capsids, elongate in form, yellow or sometimes reddish with dark markings and is found usually in great numbers on heads of timothy or other meadow grasses in midsummer. It is a common species in the Palearctic region and has been known in America for about forty years, the first record apparently being by Provancher for the vicinity of Quebec. There are several facts which point to the possibility that it may have been introduced from Europe somewhat recently and any data as to time of first appearance in any locality will be especially helpful in determining rate of dispersal. If the species is not positively known I will be glad to receive and identify specimens that may be suspected.

With sincere thanks for any information either as to presence or absence in your locality,

Very truly yours,

HERBERT OSBORN,
Experiment Station, Orono, Maine.

Prof. H. T. Fernald, of the Massachusetts Experiment Station, replied as follows:

My first note on this insect shows that I took it June 23, 1882, at Orono. I may have taken it before this, but have no note on it. I have also a note that larvæ of it were very abundant June 15th, 1883, at Orono. My remembrance of it is that it was always very abundant as far back as 1880 at least, when my observations began.

Here at Amherst it has been abundant ever since our collections were made, so far as I can learn. We get it sweeping over our grass fields, but as these fields are nearly always more or less mixed grasses, I have not been able to determine exactly which kind of grass it attacks, and in fact I have not given much attention to this insect. Here we get the larvæ in abundance about the first of May, varying with the season. Adults begin to appear early in June. I regret to say that I have no further data on this subject, but am confident that a careful examination would show a second generation here the same season at least. Whether there is a third, I do not know.

Prof. W. E. Britton, of Connecticut, wrote:

I have your letter of July 5th and wish to inform you that *Miris dolabratus* Linn. is very common in Connecticut, in fact, so common that in sweeping we do not save the specimens. I have never made a study of this species and can not tell you offhand just what species of grass it attacks.

The following records are given for Connecticut localities:

New Haven, June 16, 24, 1902 (E. J. S. Moore), June 8, 1904 (W. E. Britton); Brooksvale, Sheshire, July 30, 1902 (W. E. Britton); Branford, June 27, 1904 (H. L. Vierick); Mount Carmel (Hamden), June 10, 1908 (W. E. Britton); Stratford, June 29, 1908 (W. E. Britton); Stonington, June 14, 1906 (W. E. Britton); Wallingford, June 15, 29, 1912 (D. J. Caffery); Wetherfield, June 24, 1913 (L. B. Ripley).

Dr. E. P. Felt, State entomologist of New York, wrote as follows:

Replying to yours of the 5th instant would state that *Miris dolabratus* is a rather common and widely distributed insect in this State, attaining maturity about the middle of June. Occasionally it is exceedingly abundant, as was the case June 14, 1898, at Trenton Falls, where it literally swarmed in a field of timothy. You doubtless appreciate that unless such insects are extremely numerous and kill or nearly destroy the grass comparatively little attention is paid to them.

The following records, supplied by Dr. Felt, are from the State Museum of New York:

Albany, June 19, 1900, June 13, 1903, June 25, 1901, June 17, 19, 1899; Chazy Lake, June 28, 1913; Crane Pond, July 2, 1897; Frenchs Mill, June 14, 1902; Ithaca, June 28, 1892; Karner, June 27, 1903; Keene Valley, July 16, 1894; Mount Marcy, July 31, 1913; North Chatham, June 6, 1902; Ogdensburg, July 10, 1903; Poughkeepsie, June 2, 1903; Saranac Inn, June 27, 1913; Schodack, June 22, 1902; Trenton Falls, June 18, 1898, very abundant; Wells, July 19, 1913; Westfield, June 24, 1904.

Prof. H. A. Gossard, writing from Wooster, Ohio, sent the following data:

Replying to your inquiry regarding *Miris dolabratus* will say that it has been noted as an extremely abundant and injurious insect in Ohio meadows during several different seasons. In the latter part of June, 1907, Mr. Whitmarsh took a great many specimens which are in our collection, and it must have been rather numerous. In 1912 I noticed it in such great abundance in a mixed meadow of blue grass, redtop, and timothy on the station farm that I noted it as a species worthy of special investigation, and I am glad you are undertaking the study. I noted both larvæ and adults May 11, 1912, and there are numerous specimens in our collection taken on that date. There were also numerous specimens taken June 6, 1912. I recall noting that the punctures of the insects on the grass stems were abundant but the injury to the grass was rather indeterminable. So long as rainfall is plentiful, I would not anticipate conspicuous damage from the species, but during a dry period I apprehend that it could do as much or more damage than I have yet seen the tarnished plant bug do. I certainly have never seen the tarnished plant bug in such numbers as *Miris dolabratus* during May and June, 1912. I recall that nymphs of the species were abundant and approximately mature when the earliest grasshopper nymphs were appearing in the pasture. I have not noted the species in such numbers since 1912, but possibly I have not been collecting in places that would discover it.

Prof. H. Garman, of the Kentucky Experiment Station, wrote:

Replying to your note of July 5th I have to say that my first record of the occurrence of *Miris dolabratus* in Kentucky is May 23, 1908, when it was swept from grasses in a pasture at Lexington. I have other specimens taken at Lexington, May 10, 1913. I think if the species had been common previous to the earliest date given, I should have observed it. *Oncognathus binotatus* has been common here on timothy ever since I came to Lexington. My first records of its occurrence are in 1891, about the time it was observed by Doctor Howard.

Mr. C. A. Hart, of Urbana, Ill., replied:

Our first named specimens of *Miris dolabratus* were sent us by Dr. C. M. Weed from Columbus, Ohio; I do not know the date. Our earliest specimen, the date of which

surprises me somewhat, is a well-marked female bearing the label "Hart Coll'n" and my accession #17 in my own writing. My record shows that #17 was taken at Normal, Illinois, March 18, 1883. In 1906 a specimen was taken near Urbana. We have one from Ithaca, N. Y., in 1907, July 15, and from Doctor Nason three specimens at Algonquin, near Chicago, July 9. In 1910 Davis found it at Aurora, Ill., June 15. My first real acquaintance with the species was in 1912 when I found it about ten miles east of Urbana. The next year, 1913, it was taken near Urbana May 28 and at Mahomet, Ill., west of Urbana, May 18. Another specimen is labeled May 22 from White Heath, west of Urbana. This spring I have noticed a number of specimens in grass near the University. The almost entire absence of the species from the abundant collections of the office previous to 1906 is very good proof that it was *absent or very rare previous to that year*. With us the female is invariably brachypterous and the male is macropterous. I have no notes at all concerning its food plants or other habits. We have two nymphs, both taken near here, one May 18 and the other May 28.

Both the locality and the normal date of this record are puzzling, but Mr. Hart very kindly sent the specimen to me for examination, and his well-known accuracy scarcely admits any question as to the record, though it seems impossible that a female should have been taken alive in March.

Information from New Jersey was to the effect that no records additional to those published in the Smith list of insects of New Jersey were available. Reports from other States were mainly negative.

Prof. F. L. Washburn, of Minnesota, reported specimens from Wisconsin and Minnesota, but without definite localities or dates.

Prof. C. P. Gillette, of Colorado, states that none have been obtained in their collections in that State.

Records for the Dominion of Canada, kindly furnished from the Entomological Branch of the Department of Agriculture and transmitted by Mr. Arthur Gibson, are as follows:

Ottawa, Ontario, June 25, 1908 (Gibson), June 22, 1912 (Beaulne), August 18, 1914 (Beaulieu), September 20, 1915 (Hewitt), July 14, 1907 (Gibson); Aylmer, Quebec, June 24, 1912 (Beaulieu); Chelsea, Quebec, July 3, 1909 (Groh), July 2, 1912 (Gibson), June 21, 1916 (Gibson); Montreal, Quebec, July 7, 1906 (Beaulieu); Chicoutimi, Quebec, July 24, 1915 (Beaulieu); Youghall, New Brunswick, July 5, 1905 (Gibson); Halifax, Nova Scotia, July 11–22, 1915 (Perrin). The insect is very abundant in the Ottawa district. On June 21, 1916, many were beaten from timothy (Gibson).

PROBABLY AN INTRODUCED SPECIES

With the evidence available there seems to be good reason to believe that the species was introduced from Europe at some time during the early part of the last century, probably not earlier at best than about 1800. If we may give weight to the first records by Uhler and Provancher, it is probable that the insect was introduced in New England or Quebec or some of the other maritime provinces of Canada, perhaps equally probable for Nova Scotia, New Brunswick, or Quebec. From any of these localities the dispersal might easily have reached the other

regions concerned in the course of a few decades, although without artificial assistance its progress must have been slow.

In the Harris collection, now in the Museum of the Boston Society of Natural History, I have seen specimens which had been collected in the vicinity of Boston bearing dates of 1832, 1833, 1834, 1835. In regard to these, Uhler (*1878, p. 397*) stated that—

> This species, evidently introduced from Europe, has recently become fully established in localities where it did not exist a few years ago. In Maryland, on the edges of wheat fields, and in eastern Massachusetts on grassy low grounds, it appears in swarms. About ten years ago I first met with a few individuals near Baltimore, by sweeping the grass, etc., about the edge of a wheat field; since then they have greatly multiplied, and large numbers may now be obtained there and in similar localities elsewhere. In Cambridge, Mass., the grass is sometimes crowded with them. Specimens from Connecticut, kindly obtained for me by Mr. Edward Norton, have the antennæ yellow, and are a little more slender than usual. Both the short-winged and the fully-winged varieties occur in all the localities known to me.

Evidence in favor of the species being an introduced one may be summed up briefly as follows:

(1) *Miris dolabratus* has been a common insect in Europe for an indefinite period, covering a large area and doubtless associated with the cultivated grasses to which it seems so closely restricted here.

(2) The species was not known in America until about 1830, when it was collected by Harris, as noted by Uhler (*1878, p. 397*) and also recorded by Provancher (*1880, p. 104*), although a number of careful students such as Say, Uhler, and Walsh had given no little attention to the insects of the group to which it belongs and would almost certainly have encountered it in their work in different parts of the country where it now occurs if it had been present in any abundance.

(3) It has shown a gradual westward and southward dispersal indicated by the available records, which show that it occurred in New England in 1832, Maryland in 1868, Quebec in 1872, New York in 1887, Ohio in 1888 (?), Illinois in 1906, and Kentucky in 1908.

(4) It is adapted to certain cultivated grasses which were introduced from Europe, and its close restriction to these and apparent inability to adapt itself to native grasses even of as large forms as the cultivated ones is very significant.

(5) In the plan of hibernation of eggs in stems there is evidently furnished abundant opportunity for the transportation of eggs to distant points in hay shipped for forage or packing.

DISTRIBUTION IN MAINE

The meadow plant bug has certainly been present and abundant in Maine for many years, but except for the notes by Prof. H. T. Fernald there does not appear to have been any record that assists in determining the time of its appearance or the extent of distribution. The

Experiment Station collection contains several specimens of the adult insect, the dates recorded for Orono being July 14 and 18, 1905, and July 11, 1907. Dr. Patch published a record of its abundance in 1908 and as mentioned elsewhere, I have noted it as being abundant in 1914 at Orono. Prof. C. L. Metcalf said that the species was abundant during the summer of 1916 in late instars and adult males at Fort Kent on July 5 and 6; at Presque Isle, mostly adults, on July 8; and at Houlton as adults with few nymphs of late instars on July 9. I found them abundant at Phillips and other points between Farmington and Dallas, where timothy meadows were examined, on July 18, 1916, and also very plentiful in some old meadowland in the vicinity of Saddleback Lake on July 19 and 20. None occurred on Saddleback Mountain at any point above the level of the meadowland or the growth of the timothy and other grasses commonly occupied by the species.

It is evidently safe to assign its distribution in Maine to all parts where suitable grasses occur, and it may confidently be expected to occur during the months of June, July, and August in all old meadowland where timothy forms a part of the combination, and a search in the stems will be pretty sure to disclose the eggs of the insect during other months of the year.

ECONOMIC IMPORTANCE

While, to judge from the occurrence of great numbers of *Miris dolabratus* in meadows and the evident attack on the plants, it must be inferred that the insect causes serious injury to the crops, there appears to be little to establish the amount of loss or to separate it from that due to other species. In fact, but few of the Capsidae have been given much attention from the economic standpoint. The familiar and cosmopolitan tarnished plant bug, *Lygus pratensis*, has been known for years as a pest to many plants. In 1892 Howard (*1892*) called attention to *Oncognathus binotatus* as "a new enemy to timothy grass." Dr. M. V. Slingerland, of Cornell University, has treated the common 4-lined plant bug (*Poecilocapsus lineatus*) as a pest of currants; Prof. E. A. Popenoe, Kansas Agricultural College, has called the little *Halticus citri* (Ashm.), a garden pest of beans; and the common *Adelphocoris rapidus* has been known for many years to affect the clover crop.

Some idea of the effects produced by the meadow plant bug may be obtained by noting the enormous numbers to be found hanging to the plants and especially to the heads during the time the timothy is in bloom. Often a number cling to a single head, from three to five being not unusual. The fact that they suck the bloom doubtless means a heavy loss in seed or in weight and nutritive value of hay, although there is little external evidence of injury.

Evidence of injury based on the amount of hay per acre where the meadow plant bug is plentiful as compared with fields where it is absent,

suffers from the fact that so many different insects are present and it is almost impossible to determine the proportion of damage to be charged to each. To judge merely by the numbers present and also by the size and feeding capacity of *Miris dolabratus*, it may easily be counted among the most destructive to the crop, though it does not kill the plant by attacks at or near the root.

FOOD PLANTS

Timothy has been most commonly mentioned as the food plant of *Miris dolabratus*, and this is quite evidently the grass with which it is most commonly associated, as even where it may be found on other grasses it is usually where timothy forms a large part of the combination of species growing together.

FIG. 1.—*Miris dolabratus:* A, adult on timothy head in resting or feeding position; B, female ovipositing; C, eggs from oviduct, nearly or quite mature; D, mature egg ready for deposition; E, mature egg greatly enlarged showing membranous operculum.

I have found it commonly on timothy heads, very evidently feeding, and individuals have been carried along for several instars with no other food; hence, it is clearly a normal food supply (fig. 1). I have also found it commonly on orchard grass (*Dactylis glomerata*), meadow fescue (*Festuca elatior*), and witch grass (*Panicum capillare*) and the nymphs seem to thrive on these plants about as well as on the timothy. The meadow plant bug has not been observed commonly on bluegrass (*Poa pratensis*) or other small grasses or grasses with small seed heads, except as these are mixed with the coarser forms. While it occurs where clover is mixed with timothy and lays eggs in clover stems, it has not been observed ever to feed on stems, leaves, or heads of clover. I believe it is quite strictly a grass-feeding species, primarily adpated to timothy.

Before the grasses head out, *Miris dolaratus* is found on the stems and leaves, but the larger part of the growth of the insect occurs after timothy begins to head. The heads seem to be the favorite point of attack. The insects cluster on the heads sometimes in numbers to a single head and thrust their beaks down into the flowerets, evidently drawing their

food from the tender parts of the blossom or from the forming seed. I have seen a female thrust her beak down in the flower of witch grass, piercing the glumes, or insert her beak between and down into the anthers, penetrating them and causing them to burst, and probably sucking juices from the ovules.

Slosson's (*1894*) record for Mount Washington above 5,500 feet is for adults, and Mr. C. W. Johnson informed me that many insects capable of flight are carried up by air currents and found at altitudes much above that of their food plants.

SYNONYMY

The abundance, wide distribution, and variability of the meadow plant bug may be inferred from the many names which have been applied to it by different writers. No less than 13 specific names have been given to it, and with the various generic combinations used this number is still increased.

For the detailed statement of the synonymy I am indebted mainly to the exhaustive catalogue of Oshanin (*1909, p. 778*), whose records are almost exclusively European. A more complete bibliography is given by Van Duzee (*1917*). Its synonymy according to Oshanin is as follows:

MIRIS FAB. REUT.

Miris Fabr. S. R. p. 253 (prt.); Reut. Rev. Syn. p. 243; Hüeb. Syn. Blindw. 1, p. 33 et 63; *Leptopterna* Fieb. Cr. Phyt. p. 302; Eur. Hem. p. 63 et 244; Reut. Gen. Cim. p. 9; Rev. cr. Caps. 2, p. 13; *Lopomorphus* Dgl. Sc. B. H., p. 293. *Lopus.* Herrick Schaeffer. Wanz. Ins. III., p. 35.

dolabratus Lin.

Cimex dolabratus L. Syst. Nat. ed. 10, p. 449 [1758]; ? *Cimes frumentaruis* Poda Ins. Mus. Graec., p. 60 [1761]; *Cimex riparius* Scop. Ent. carn., p. 135 [1763]; *Cimex laevigatus* Deg. Mém. 3, p. 292 [1773]; *Cimex lateralis* Fabr. Gen. Ins., p. 300 [1776]; ? *Cimex deses* Müll. Zool. Dan., p. 108 [1776]; *Cimex antenni-rectus* Goeze Ent. Beitr. 2, p. 267 [1778]; *Cimex V-flavum* Goeze ibidem, p. 279 [1778]; *Cimex porrectus* Geoffr. in Fourcr. Ent. Par., p. 206 [1785]; *Cimex recticornis* Gmel. Syst. Nat. ed. 13, p. 2185 [1788]; *Miris abbreviatus* Wolff Wanz. f. 110 (♀) [1802]; *Miris lateralis* Wolff Wanz. f. 109 (♂) [1802]; ? *Miris picticeps* Curt. Brit. Ent. 15, t. 701 [1838]; *Miris dolabratus* Hhn. W. I. 2, p. 75. f. 160 [1834]; Flor. R. L. 1, p. 437; Reut. Rev. syn. no. 209; Hüeb. Syn. Blindw. 1, p. 66; Atk. Cat., p. 34; *Lopus id.* H. S. W. I. 3, p. 45, f. 261 et 262 [1835]; Mey. Caps., p. 38; Shlb. Mon. Geoc. p. 88; Kbm. Caps., p. 196; *Leptopterna id.* Fieb. Eur. Hem. p. 245 (prt.) [1861]; Reut. Rev. cr. Caps. 2, p. 15; Saund. Synops. 2, p. 262; Prov. Faun. Ent. Can. Hém., p. 104 [1886]; Saund. Hem. Het. Br., p. 227, t. 20, f. 10; *Lopomorphus id.* Dgl. Sc. B. H., p. 297 [1865]; Uhler, Bost. Soc. N. H. XIX, p. 397. 1878; *Miris Belangeri* Prov. Natur. Canad. 4, p. 78 [1872].

DIMORPHISM

The species occurs in two distinct forms of females, a long-winged and a short-winged form; but only one form of male, the long-winged, has been observed. The short-winged form of female is by far the most abundant; and as this form is entirely unable to fly, and therefore is very

definitely restricted in its migration, it is of special interest to note that it must be the form which produces pratically all of the eggs.

In the short-winged form the elytra reach only to the fifth abdominal segment leaving a large part of the abdomen, especially when engorged with eggs, conspicuously exposed. While there is some variation and occasional intermediate forms, there is a great preponderance of individuals with elytra uniformly about 5 mm. long and with the wings still shorter. None have been seen with the wings entirely aborted.

The relative proportion of eggs produced by the two forms will be discussed under the head of egg production, but it may be said here that from collections made during the summer of 1916 it appears that only about 10 per cent of the females are long-winged and that these produce a much smaller number of eggs each than the short-winged forms.

The biologic significance of the dimorphism can hardly be entered on here. It is, however, distinctly similar to what occurs in many other of the Hemiptera and doubtless depends on some fundamental factors in food supply, seasoned migration, or other adaptation.

It offers many interesting biological problems for investigation and naturally presents some most essential elements in the consideration of general control.

LIFE HISTORY

The eggs hatch in May or early June, the time being determined in part by latitude and season. The exact date of hatching at Orono was not observed, as nymphs were already abundant at the time of my arrival on June 12, and as the season of 1916 was exceptionally late, it is probable that the average date of hatching would be the last week in May. Young nymphs continued to appear until about June 25, but none hatched after July 1. The first adults appeared on June 16 and were abundant by June 26.

Evidently the adults feed for some time before mating, as the first matings observed were on July 8 and 10. The eggs, however, develop rapidly when the insects reach the adult stage, since fully developed eggs in large numbers, 50 to 60 to the individual, were dissected from the females, the first one dissected, on June 30, containing 30 eggs fully formed, as well as others in an immature state. Another, dissected on July 8, contained 69 developed and a few immature eggs.

These dissected eggs were of special interest, as they might furnish the clue to a later determination of place and method of oviposition, as the peculiar strongly curved neck and large membranous expansion over the head naturally suggested some rather unusual mode of placement.

The nymphs cling closely to the plants and while they pass readily up and down the stems and doubtless shift from one plant to another there is no extended migration, probably no movement providing for any dispersal. When molting, they cling to the plant and, as with other

insects generally, the skin splits along the middle line of the back and the body and legs are gradually withdrawn and the increase in size and resumption of color takes place in a short time.

Five distinct stages of the nymphs are recognized and this seems to be the general rule for the Hemiptera, being the number noted in a large number of the species which have been reared through the nymphal stages. These will be described in detail in a later paragraph (fig. 2).

No single individual has been carried from the first instar through to the adult stage, but numbers have been carried from two to four of the instars in confinement and under observation so that it is possible to give a connected series of stages from the smallest found to the adult

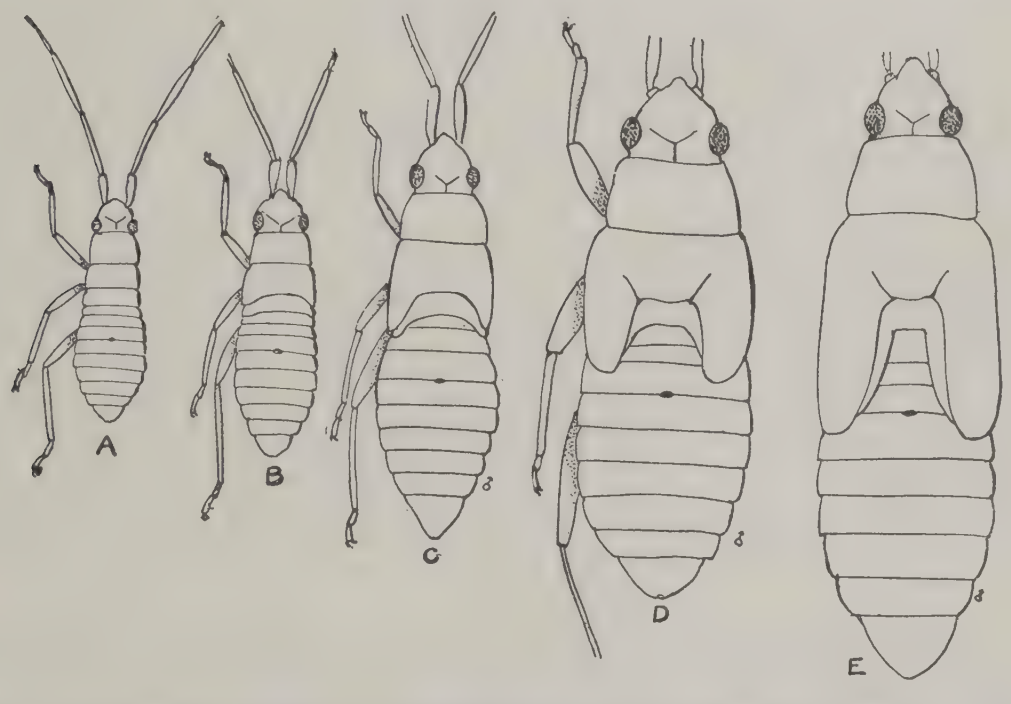

FIG. 2.—*Miris dolabratus:* Nymphs showing relative size of body and development of wing pads. A, First instar; B, second instar; C, third instar; D, fourth instar; E, fifth instar. Note also position of dorsal gland orifice between segments 3 and 4. (Original.)

form. The time occupied in the different stages has run from 5 to 8 days, averaging 6 to 7 days, and the total period of development from hatching to adult stage must be about 30 to 35 days.

The principal changes are in the increase in size and in the growth of the wing pads, which are entirely wanting in the first, appear as faint enlargements of the mesothorax in the second instar, are fairly distinct on both mesothorax and metathorax in the third, extend to the second abdominal segment in the fourth and on to the middle of the fourth segment in the fifth for the female and to the base of the fifth segment for the male. There is considerable irregularity in development, as instars 1 to 4, and probably 5, with adults were taken on June 23.

In the fourth and fifth instars the sexes are easily distinguished, males being slightly narrower, the abdomen with more parallel sides, and the

genitalia being seen in outline through the semitransparent walls (fig. 3, A, B).

With the final molt the wings expand rapidly, and the distinction between males and females and the dimorphic forms of females become clearly marked.

The adults remain quite constantly on the grass heads and evidently feed for a number of days before mating or egg laying begins.

The insects cling to the stems or heads of grass quite firmly, the tarsi, which are quite long, being evidently well fitted for securing a firm hold to the parts of the grass. They seem to obtain the best hold on the smaller stems, 1½ to 2 mm. in diameter. On the timothy heads the tarsi catch in the florets, which seems to give them a very firm foothold, as they are not easily dislodged. They could doubtless cling to the heads easily while the hay is being handled.

On the grass heads they are more commonly found with their heads directed upward, and they show some tendency to mount to the highest

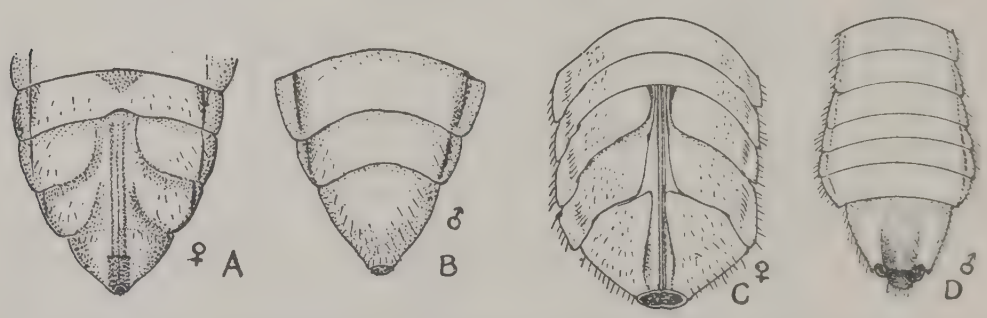

FIG. 3.—*Miris dolabratus*, genital segments: A, Female; B, male of fifth instar nymph; C, female; D, male of adult. (Original.)

point; but they often stand head downward and pass up and down the heads and stems freely. The position with the head upward appears to give them an excellent position for probing into the florets with their beaks. It was noted that in egg laying the females selected small stems of grass, 1 to 1½ mm. in diameter, while in clover stems as much as 2½ mm. diameter were used. Probably the rough or softer stem of clover serves as a good foothold, though the tarsi are too small to clasp around it.

The insects are not very conspicuous on the grass, even where plentiful, and may be somewhat protected by their form and coloration, especially from a little distance. At close range they are disposed to dodge behind the grass when disturbed, and they can move with considerable celerity.

The proportion of the sexes and the two forms of female is of interest and possibly of economic significance, since it bears very directly on the ability of the species to spread from the fields in which they hatch.

In the ordinary field captures the short-winged females were in the majority, and in captures with special effort to obtain all forms they were usually somewhat more numerous than males, a fact that may be

due to the greater activity of the males which enables them to avoid the net. The disparity is not so great, and a fairly equal proportion of males and females may be assumed. For the macropterous and brachypterous forms of the female, however, there is very evidently a decided disproportion, amounting in the captures at Orono to about 1 to 9—that is, 90 per cent of the short-winged to 10 per cent of the long-winged. Of 125 collected on July 11, 51 (40.8 per cent) were males and 74 (59.2 per cent) females. Of the females, 72 were short-winged and 2 long-winged.

This disproportion seems to be still further magnified by the greater number of eggs developed in the short-winged forms.

There is some reason to think that the long-winged females develop slightly earlier than the short-winged ones, and possibly there is some scattering of these and an earlier egg deposition; therefore observations to correct or verify this point are desirable.

As usually found in the field, the brachypterous forms were much more evident and undoubtedly constitute the main source of egg production. They usually show much greater distension of the abdomen, and are full of well-developed eggs at time of mating. They are so heavy they would probably fly with difficulty even if the wings were not aborted. As it is, they can not fly at all, make no attempt to use the wings, but drop or flutter helplessly to the ground when thrown into the air; hence they must necessarily lay their eggs in the immediate vicinity of the place where they have hatched and grown. No mating of long-winged females was observed, but this is not strange and does not warrant the assumption that they are unfertilized.

EGG PRODUCTION

The dissection of a number of individuals when fairly mature as well as the number of eggs deposited by individuals under observation indicates an egg production of from 60 to 70 for each brachypterous female. One dissected macropterous female contained 60 eggs, but all others had from none to 15. Of those dissected on July 11, 27 contained a total of 58, 21 had none (it is barely possible they had completed oviposition), 4 had 10 or more, the highest being 15 each.

If these figures can be taken as at all representative of average conditions, it means that on the basis of an average of 70 eggs per individual for the brachypterous and of 2 for the macropterous form, and assuming that 10 per cent were macropterous, the macropterous would produce but one three hundredths of the eggs, which would be an almost negligible number as affecting dispersal and would raise some interesting biological questions concerning the survival of the brachypterous form. However, as already hinted, these figures must be considered as representing a very limited period and were obtained before we had sufficient acquaintance with the species to secure proper checks.

MATING

The preliminary steps in mating have not been observed, but in a number of cases males and females in copulation have been kept under observation for several hours. The usual position is for the male to be at the right side of the female with the legs of the left side clasping her body, the forelegs crossing the forward part of the body and the others disposed across the thorax and base of the elytra. The right legs remain free and may be used slightly in clinging to adjacent objects, but the female alone may cling to the grass head or stem. The tarsi of the left legs of the male are held against the margin of the female's body and apparently kept in one position for the entire period of mating. The abdomen of the male is bent under the female with its dorsal face uppermost and the genital organs inserted at the base of the ovipositor.

In one instance a pair taken on July 10 about 7.40 a. m. and carried on the grass stem for about a mile remained in copulation until 9.40 in spite of considerable handling. After separation they showed no inclination to subsequent mating. The male died on the third day following, and the female began ovipositing in about 24 hours after mating, certainly then and possibly earlier. Other observations confirm the view that mating lasts for several hours, and no evidence has been found that either males or females mate more than once.

Considering the tenacity of their hold and the tendency to cling closely to the grass while mating, it is possible that methods for capturing them might be more effective at this period. It is quite possible that they might be carried with hay at this time more easily than otherwise, and if scattered in favorable situations serve to provide for wider dispersal

EGG DEPOSITION

The process of egg deposition is interesting and has been watched repeatedly with females confined in glass tubes, but it has not been observed directly in the field. The females in the field evidently succeed in keeping out of sight or perform their egg laying with such rapidity that there is little chance of finding them at work. It was only after watching them in confinement and learning how the eggs were laid that we succeeded in discovering the eggs in stems in the field where the insects had been abundant.

When about to oviposit, the female seeks a suitable place on the stem, more frequently with the head upward, but often in the reverse position, and explores the stem carefully with the beak. She appears to make a slight puncture with the beak then draws the abdomen at a sharp curve up under the thorax and places the tip of the ovipositor on the spot where the beak has rested. The ovipositor is withdrawn from its sheath and stands at nearly right angles to the genital segment and the insect with all legs clasping the stem and evidently strained very tensely begins

a swaying movement forward and backward working the tip of the ovipositor slowly into the stem. If unable to start it promptly, she may shift her position and reinsert the beak, then begin again with the ovipositor. When the point of the ovipositor has been thrust in, evidently at the point where it has penetrated the outer wall of the stem, the body is pushed forward and the ovipositor pushed strongly downward and backward till it is embedded its full length. A few contractions of the abdominal segments serve to slip the egg along the ovipositor, a scarcely visible operation from the outside, and the ovipositor is quickly withdrawn. The slit in the stem closes up so as to be entirely invisible. The egg is held by an operculum. The insect takes a short interval of rest or may renew the process almost immediately, eggs being laid at the rate of one every minute or minute and half to two minutes. On the withdrawal of the ovipositor, she feels with her beak the point where the egg was inserted, moves forward a trifle, and again feels the surface with the beak, apparently relaxes a little and then, rubbing the fore tarsi together and holding them free, vibrates the joints as if to limber up after the severe exertion of forcing the ovipositor into the stem, and proceeds to lay another egg. About 20 eggs have been laid in the course of half an hour, and this is about the highest number usually found in a series in stems collected in the field.

That eggs are laid on different days was shown by one female which laid about 20 one afternoon between 4 and 5 o'clock, 11 on the following day at about the same hour, and 6 the third day between 4.40 and 4.50 p. m. She may have laid others when not under observation, as 52 eggs were later counted in the stems in which she had oviposited, and these were pretty surely all laid by this one female. As she was dead on the morning of the sixth day after the first oviposition had been seen, she may have laid eggs possibly on four or five days, but certainly most of them laid after she was under observation must have been deposited during three days. It is rather striking that the eggs should be laid on successive days at so near the same time of day. In other instances oviposition was observed in the morning, but the time of day may be fairly uniform for each female. This long interval affords the insect an opportunity to rest, probably to feed, and may also be associated with the maturing of the eggs or their adjustment in the oviducts.

The insects disappear rapidly after the egg-laying period, so quickly in fact that we were much puzzled as to their whereabouts. An examination within a day or two, especially after the grass had been cut, would show scarcely an insect where they had been numerous; therefore a special effort was made to follow them after the mowing. The males, of course, could fly readily, but they show a tendency to cling pretty closely to the grass and might be carried from the fields with the hay. The females cling still more closely to the grass, the short-winged ones making no attempt to fly, and the long-winged ones having apparently

very little disposition to travel in that way. After the cutting of the grass, the females would be found running about over it, no males nor long-winged females being observed. Mr. Fletcher reports close watching of one female for an hour and a quarter. She ran about over the cut grass, but did not attempt to get beneath it nor hide in any way, except when a sudden movement by the observer took place. Then the insect would dodge behind or under a grass stalk, where it would remain for a few minutes. It was not observed to feed during the time it was watched. In another field, an old meadow, a careful examination was made, Mr. Fletcher reporting that—

> A course was taken from the outside swath to the inside one so that grass cut for about two hours and freshly cut grass was examined. Females were found easily upon the top of the grass; but no males. Two long-winged females observed acted to all appearances like the short-winged individuals, making no attempt to fly. The females made no attempt to seek shade, though the sun was high.

However, the insects ordinarily cling to grass heads in full exposure to the strongest heat of the sun.

After the hay has been raked together, it is practically impossible to find insects in the stubble, however, they may be found for a time on the haycocks, but not down in the hay. Some of them may very probably be carried in with the hay to the haymows, but are not to be found by an ordinary search.

The most plausible explanation for this very rapid disappearance is that the insects die shortly after egg deposition, and that as most of the females had oviposited before the grass was mowed, they died off rapidly afterwards, and their shriveled bodies became difficult to find or were disposed of by ants. Their bodies are very fragile, and easily broken to pieces when dry; hence, a few days of dryness, or possibly a heavy rain, may destroy nearly all traces of the dead bodies.

Those that have not deposited eggs can evidently carry this on readily in the stubble, as it has been determined that the great majority of the eggs are placed near the ground.

It is entirely probable that females may be carried in with the hay and deposit eggs in the stems after the hay has been stored. Where the hay has been cut early or before the time of egg deposition, this is likely to occur; but from the apparent efforts of the insects to keep above the mass of hay it seems probable that the majority of those covered deeply would fail to oviposit. It is doubtful whether they could oviposit in dry stems, and even if they did, the fate of the eggs would seem to offer little opportunity for the serious dispersal of the insects, except where shipped to a distant locality.

It is conceivable that, considering the evident toughness of the egg-shells and their ability to resist adverse conditions, the eggs might pass through the alimentary canals of horses or cattle undigested and uninjured, and later possibly be carried back to fields in the form of manure;

but such a transfer, even if possible, must have little, if any, practical importance, considering the normal condition of the great mass of eggs being placed near the ground and protected for their period of development in the stubble.

PLACE OF DEPOSITION

In order to determine as certainly as possible where the majority of the eggs are deposited, counts were made of a number of stems of the grasses on which the adults commonly occur and from a field where the insects had been present in large numbers during the present summer. One hundred stems each of timothy, orchard grass, meadow fescue, and witch grass were selected from a point where the insects must have been abundant and where eggs should certainly be plentifully found. Out of this number two timothy stems were found with eggs, one of meadow fescue, and one of orchard grass, but the witch grass had none. An additional 25 stalks of timothy were split, and many stalks were examined and slit part or all of their length without finding any eggs in the upper part of the stem. In no case were eggs found beneath leaf sheaths or in stems where it would have been necessary to puncture through the leaf sheaths to reach the stem.

It appears very clear, therefore, that the great majority of the eggs are placed in the stems of plants near the ground and below the level of cutting, so that but very few can be carried out of the field with the hay in cutting or harvesting. This makes very clear the presence of immense numbers of insects in meadows which are regularly mowed and from which the hay is removed, and emphasizes the effect of the short-winged condition and inability to fly of the greater proportion of the females. However, a sufficient number of eggs may be placed in the stems above the height of the stubble or be raked up in stems from the ground and carried in with the hay to provide for limited dispersal and account for the transportation of the species from one region to another (Pl. 12).

DESCRIPTION OF MIRIS DOLABRATUS

No very satisfactory description of *Miris dolabratus* is available in the accessible textbooks or manuals.

The adults are about two-fifths of an inch long (9 mm.), rather slender, with long black antennæ which are thickest at the base, the head is rounded, set fairly close to the thorax, which widens behind; and the wings lie nearly flat on the back, are narrow, and have nearly parallel sides, extend to or slightly beyond the tip of the abdomen in the males and long-winged females and to the fifth abdominal segment in the short-winged females. The color is yellow or yellowish gray with dark markings, which form two rather indefinite stripes on the pronotum and elytra. The antennæ and legs are black, with yellowish bases or yellow with black hairs and spots.

The nymphs are yellow marked with black, the general color being quite dark in the early instars and becoming lighter with the successive molts.

DESCRIPTIONS OF EARLY STAGES

THE EGG

The egg of *Miris dolabratus* is of quite unusual shape and shows a quite remarkable adaptation to its anchorage in the stems of plants. It is of elongate-oval and strongly curved especially near the head or attached end, which expands slightly from a constriction in the curved part and is surmounted by a large flat chorionic membranous expansion or operculum, the width and length of which is nearly one-fourth that of the egg itself (fig. 1). The egg is shining, polished with a dense chorion, and when placed in the stem with the operculum held firmly in the wall stands out into the hollow of the stem. In clover stems they may be embedded partly in the pithy layer lining the stem. The eggs are fully developed in the ovaries soon after the adults mature and show fully the enlarged opercula. The position of micropile and the route of entry for the spermatozoa is undetermined, though it would seem almost necessarily through the operculum. Egg length, 1.35 mm., diameter 0.25 mm. (fig. 1).

FIRST INSTAR

The smallest individuals found, and evidently first-instar individuals partly grown, were 2.25 to 2.50 mm. long. The color is quite dusky or blackish, the antennæ and legs being entirely black, except a slight pale portion of membrane at joints. The head above is blackish with yellow area next the eye; beneath it is yellow. The thorax is yellow, with a broad black stripe on either side; or it may be black with the median and the marginal line yellow. The abdomen is brown, the lateral stripe being yellow, and an oblique patch on each segment is yellow. Beneath the abdomen is brown, with the hinder border of segments yellow.

The antennæ have a total length of 2 mm.; the second and third segments are equal in length, slightly longer than the fourth and twice as long as the first, the fourth being about as thick as the third. The beak extends to the hind coxæ and is thick.

SECOND INSTAR

The insect in the second instar resembles the first in color, being dusky though of a trifle lighter color and showing more of the greenish gray of the later stages. This color becomes more pronounced as the insect grows during the instar, especially for the margins of the thorax and abdomen. The wing pads are very slightly indicated as blunt lobes on the hinder borders of mesothorax and metathorax. The antennæ are

distinctly longer, the second segment being longest, with the third a little longer than the fourth. The beak reaches the base of the hind coxæ. The length is 3 to 3.5 mm. and length of antennæ 3 mm.

THIRD INSTAR

The third-instar individual is distinctly lighter colored, and the pattern of marking is more like that of the later stages. The wing pads are distinctly indicated, those of the metathorax extending well onto the base of the second abdominal segment and those of the mesothorax extending well back on the metathorax pads to a line with the base of the second abdominal segment. The antennal segment, too, is nearly one and a half times the length of the third, the third twice the length of the fourth and three times the length of the first. The beak reaches between the mid and hind coxæ. Length, 5 mm. Total length of antennæ, 4.5 to 5 mm.

FOURTH INSTAR

Lighter in color, greenish border of thorax and abdomen broader, first and second segments of the antennæ, except at apex greenish; legs except tarsi greenish, hairs black; antennal second segment about one and one-half times as long as the third, the third three times as long as the fourth and first, fourth and first about equal. The wing pads are considerably enlarged, the mesothoracic pads extending over and beyond the metathoracic pads and to the middle of the third abdominal segment. The beak reaches to the hind border of the middle coxæ. Length of body, 6 mm.; of antennæ, 6 to 6.5 mm.

FIFTH INSTAR

The individual of the fifth instar is light gray-green or yellowish green with black or fuscous patches and stripes forming two nearly continuous stripes over the wing pads and abdominal segments, paralleling the margin, and a median double row of spots on abdomen; antennæ greenish, tips of first and second and all of third and fourth segments blackish, legs greenish, tarsi black. Antennal segment two three times as long as the first, two and one-fourth times as long as the fourth, and about one and two-thirds times as long as the third. Length of body, 6.75 to 7.25 mm.; of antennæ, male, 6.25 mm. The beak extends to the base of the middle coxae.

The characters that seem of special service in recognizing the various instars are the comparative lengths of the antennal segments and the development of the wing pads. While these are subject to slight variation in different individuals, they seem fairly constant, and the descriptions and figures have been made from what seemed to be representative specimens. The orifice of the dorsal gland between third and fourth segment, while not furnishing distinctive characters of the instars, is a good landmark for locating the abdominal segments.

The adult antennæ are much longer proportionately, and this elongation is due mainly to elongation of first and second segments, the first is one-third of the second, the second nearly four times the fourth and nearly twice as long as the third (fig. 4).

NATURAL ENEMIES

Among the natural enemies or checks of the species may be counted the gray-damsel bug, *Reduviolus ferus* (fig. 5), which is a very common and widely distributed species throughout the northern part, at least, of North America, and in fact the holarctic region. Webster and Mally (*1897, p. 41*) states that *Coriscus ferus* was observed to attack *Leptoterma dolobrata* L. which was feeding quite abundantly on timothy heads about East Cleveland, Ohio, on June 28, 1896.

Fig. 4.—*Miris dolabratus:* A–F, Antennæ of nymphs: F, antenna of adult male drawn to same scale and showing relative lengths of segments. (Original.)

This species has been found in Maine associated with *Miris dolabratus* as well as with the leafhoppers, and it is probable that it may feed on either with equal avidity. While it has not been seen feeding on the mature *M. dolabratus*, it is pretty certain that it will feed upon the larvæ, as its fondness for leafhoppers in both the nymph and adult stages has been proved by repeated observation. Direct observation of the insects attacked is difficult, as it is almost impossible to follow them in their movements in the field. Even when offered a variety of food in confinement it is difficult to determine their selection in the species offered, as they very seldom make their attacks on the insects while under observation, and it is left to infer from the numbers killed how ready they are to prey upon different kinds. We have had them live and thrive in confinement and progress to the adult stage on a diet of *M. dolabratus* and also on a food supply of leafhoppers, and it is probable that they will eat a variety of small insects, the particular kinds being determined rather by the available supply than by any definite choice on the part of the bug.

They are able to survive considerable periods without food, and their development is doubtless affected either by abundance or scarcity.

The appearance of the different instars of the early stages is not at all uniform, as individuals of several different stages, as well as adults,

may be found at the same time in the early part of summer. Thus, a first-instar individual was taken on June 27 and a second-instar on July 5, a third on July 6; but fifth-instar individuals occurred on June 26; one changed to adult on June 30, and the other fifth-instar individuals were taken on July 6 and 13. Mr. Fletcher collected 10 nymphs, last instar, and 33 adults, 9 males and 24 females. During the latter part of the summer only adults are found, so it appears that the early stages must be passed during spring and early summer. During the earlier part of the summer fifth-instar individuals were taken much more commonly than the earlier stages, and it would seem that they remain in this stage longer than the earlier instars, or else they are in position for a more ready collection. Confined and furnished occasional leafhoppers as food, they have been carried for a period of seven days in this stage.

Adult females contain well-developed eggs in late summer, and while there is no probability of a second generation, at least in the latitude of Orono, it appears probable that eggs are laid in autumn to hatch the following spring.

The smallest individuals found and evidently of the first instar are of a very delicate, almost transparent whitish color, with a length of 2.5 mm. and a width of thorax of about 0.6 mm. The hind tibiæ are 1.5 mm. The antennæ have four joints: First thickest, second slightly longer than the first, the third the longest, a trifle longer than the second, the fourth about equal to the third. The eyes are red. A conspicuous red line is seen on the thorax and abdomen, with a dusky stripe at the side of the thorax (fig. 5, A).

The second-instar individuals have a length of 4 mm., with antennæ of 3 mm. A conspicuous dark-red median line runs from the head to the tip of abdomen. A broad yellow stripe occupies the middle of the body, bordered by a dusky, irregular stripe each side. Legs light yellow spotted with black, tarsi black at tip. The wing pads very slightly indicated at the outer angles of mesothorax and metathorax (fig. 5, B).

The third instar has a length of 4.5 mm. and is a little thicker than the second. The red line along the median dorsum is conspicuous, but somewhat broken and at places very slender. The antennæ are dark except tip of first and basal four-fifths of the second segments (fig. 5, C).

No specimens considered as representing the fourth instar have been seen.

The final observed nymphal instar and apparently the fifth has a length of 6 mm., a width at base of abdomen of about 2 mm., and is elongate-fusiform in shape. The head is slender and the eyes globose. Antennæ slender, the first joint thickest, minutely hairy, second and third about equal in length and slightly longer than first or fourth. Forelegs with the femora enlarged, middle femora nearly as large as the fore, the hind femora slender. The beak is 3-jointed and reaches

to base of the first coxæ. The color above is olive, with a central spot on segments 3 and 4 and the margins of the abdomen yellow or salmon-colored, tinged with orange. A dark-red or red-brown median line runs from the head to end of abdomen interrupted on base of meso-thorax and

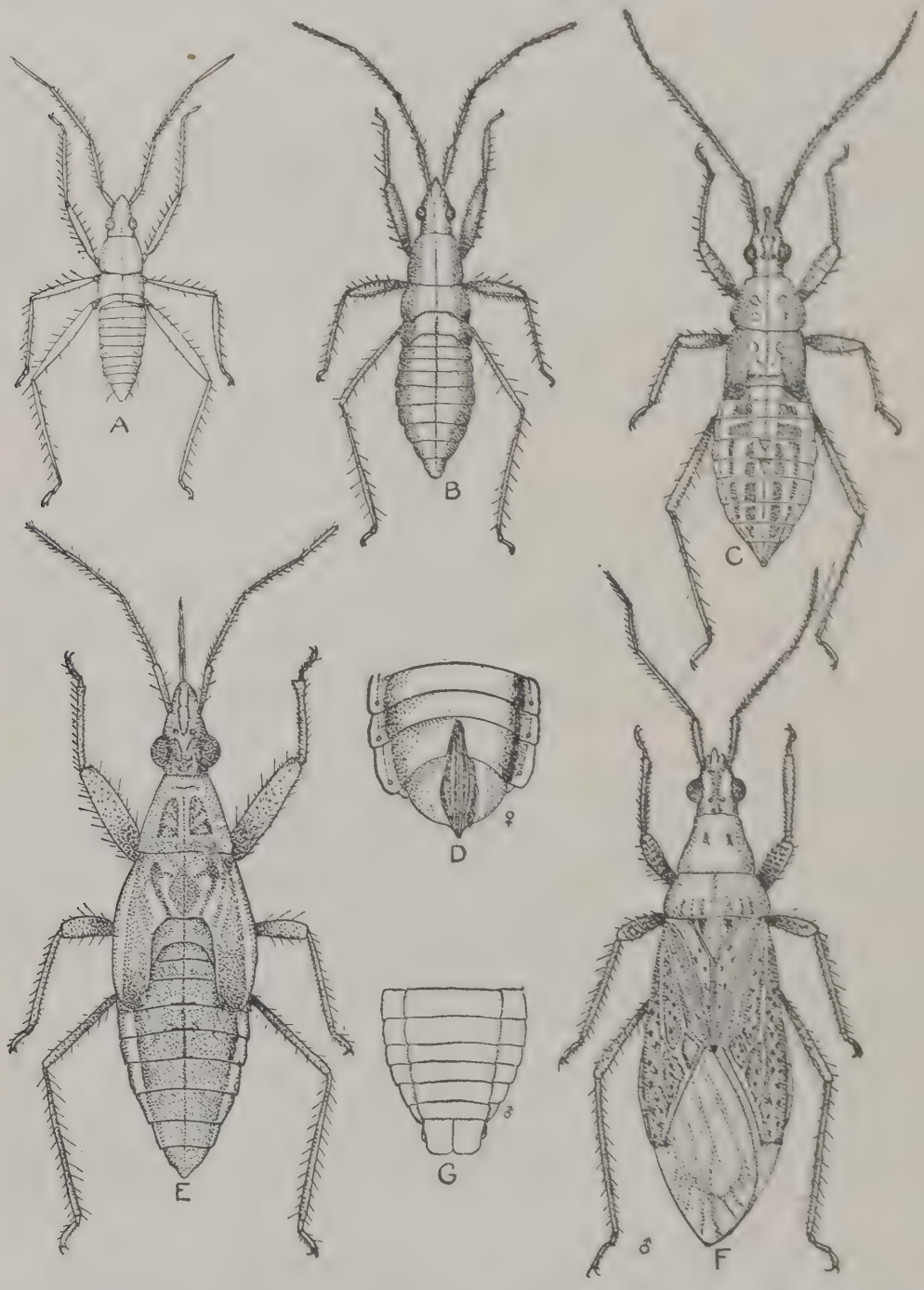

FIG. 5.—*Reduviolus ferus* L.; A, first instar; B, second instar; C, third instar; D, genital segments of female; E, fifth instar; F, adult male; G, genitalia of male.

at abdominal articulations. There are dark spots of various designs on the head, prothorax, mesothorax, and wing pads. The wing pads are dusky at tips; legs with black dots; tarsal tips black. Yellow spots occur on head, thorax, and a marginal series on abdomen in outer part of the olive

area. Beneath olive and yellow, the former mainly in form of a broad median stripe including some yellow spots. The pectus is mainly yellow, the abdomen yellow (fig. 5, E).

Reference has been made to the record of Leonard (*1916*) of rearing *Phorantha occidentis* from *Miris dolabratus* in New York. A larva, apparently a tachinid, was obtained issuing from a nymph of *M. dolabratus* at Orono, June 17, 1916, but it failed to mature. On July 18, 1916, a nymph which issued from *M. dolabratus* buried itself promptly in the earth and from this an adult emerged on July 29. This larva issued from the anterior part of the abdomen under the wings, while the host was still alive. The species has not been determined, but is evidently closely related to the species bred by Leonard.

Spiders are undoubtedly quite efficient enemies of the plant bugs, but they dispose of their prey so completely that little is left as evidence of their work. Mr. Fletcher found an individual of *Miris dolabratus* encircled by a spider's web in which it had evidently been captured and enmeshed. In some unpublished records made by Mr. Sherman Bilsing in Ohio a number of different species of spiders are credited with feeding on this species, along with many other capsids.

The extent to which birds, toads, and other animals may serve to reduce their numbers is open to study.

The Entomophthorae may be considered as potential checks, but so far no extensive destruction of *Miris dolabratus* has been noted by them. A dead specimen infested with fungus was collected by Mr. Newman on June 28, 1916. This was sent to the Bureau of Entomology and referred by Dr. Howard to Mr. A. T. Speare. His report is as follows:

> The single specimen of *Miris dolabratus* infected by a fungus closely related to the above (*Entomophthora aphidis*), but, owing to insufficient material, I would not venture to assign it anything but the generic name Entomophthora. Both contributions are very interesting, the latter especially forming, I believe, a new host for this group of parasites.

CONTROL MEASURES

While the main efforts in the studies during the summer of 1916 have been directed toward solving a number of puzzling questions as to the development and habits of the species, and while some definite experiments are needed with certain measures that may be suggested on the strength of the facts learned, there is certainly a very definite basis established for certain kinds of treatment that should do much to reduce the numbers of the insect.

The most fundamental point determined with reference to the insect in connection with control is doubtless the fact that the eggs are deposited in the stems of plants in the fields where the insect has been present. This, in connection with the fact that the great majority, probably 90 per cent of the females, are wingless, means that we know that practically all the eggs deposited in midsummer are in the old timothy meadows

and that any measure which will destroy them in this location will have immediate effect in preventing further loss from this source.

It is very evident that plowing under and planting of the field to a different crop will absolutely prevent further injury from the stock of insects established in any old meadow, and therefore rotation, where this is practicable, may be counted a certain remedy for the field concerned. However, for the protection of adjacent fields or in order to exterminate the insect as completely as possible the borders of the fields and the fence rows usually supporting a considerable growth of grass should be remembered and, for the disposal of this insect, should be plowed as closely to the border as possible or burned over when the grass is dry, so as to destroy the eggs as completely as possible.

Where rotation is impracticable or undesirable, it will be more difficult to obtain complete eradication, and careful tests of treatment, based on the habits of the insect, are necessary to determine the most successful methods.

It is clear that burning over of meadows if sufficiently dry in autumn or early spring so as to destroy the eggs would be very effective, but there are, of course, many objections to this treatment so that it can not be urged as sufficient. In some seasons probably there would be no time when the grass would burn sufficiently close to the ground to destroy any large part of the eggs and there is the danger, if burned too deeply, that the stand of grass will be injured. This method, especially for the conditions prevailing in Maine, does not seem to promise much. Where burning is practiced, it should assist. It would be worth while to compare results in field so treated.

Early or late cutting of the crop may have some effect on the number of eggs laid in a field, an early cutting, before the insects are mature, for example, depriving them of their usual form of food, the heads of grass, may reduce egg deposition, but whether to such an extent as to warrant any special change in the usual practice as to time of cutting can only be determined by further study.

The application of any form of insecticide or of special kinds of fertilizers does not seem to offer any very practical relief, and the use of hopper-dozers or mechanical devices for their capture have not been tested; nor do they have much promise.

Finally there is the important consideration of the spread of the insect into adjacent fields or farms or to more distant points, and for this the facts obtained furnish a very sure foundation for effective control. Since practically the only opportunity for such wider distribution is by carriage of hay, the disposal of any such material introduced where the insect is not present in some way so as to avoid scattering the eggs where they can hatch where suitable food plants will be available for their subsistence will serve to exclude them.

SUMMARY

(1) *Miris dolabratus* has been a conspicuous insect in timothy meadows in portions of the eastern United States during the past 40 years and now has a distribution as far west as Illinois and Minnesota and south in the Mississippi Valley into Kentucky.

(2) It is believed to be an introduced species, coming from Europe with timothy hay or other large-stemmed grass shipped for forage or packing some time between 1800 and 1825.

(3) It feeds upon cultivated grasses, especially timothy, orchard grass, and meadow fescue, and when abundant must seriously affect the value of the crop.

(4) It is a dimorphic species, there being two forms of females, a long-winged and a short-winged form, the latter being far more plentiful, about 90 per cent.

(5) The species hibernates in the egg form; hatching occurs about May 25 to June 10 in Maine; and the nymphs pass through five instars of about six or seven days each, adults occurring from early July, mating and laying eggs from July 10 to August 1 for the short-winged forms necessarily in the fields where the females have developed.

(6) The eggs are laid in stems of grass or clover in fields where females have grown, being thrust through the wall of the stem and held by an expanded cap which is firmly held by the walls of the stem, the egg being protected in the hollow of the stem and in this position remain for at least eight or nine months before hatching.

(7) Measures for control so far evident and based on habits determined will consist especially of rotation, with probably some advantage from burning, early cutting, pasturing heavily in fall, and possibly by mechanical devices for capturing the nymphs or adults.

(8) The spread of the insect should be prevented by care in the disposition of timothy hay moved to a distance. No hay from an infested district should be allowed to be scattered in or near meadows in localities where the insect is not already present.

(9) Natural enemies consist so far as at present known of spiders, the predacious damsel bugs, *Reduviolus ferus*, a tachnid fly, *Phorantha occidentis*, and an undetermined species and a species of fungus, *Entomophthora* sp.

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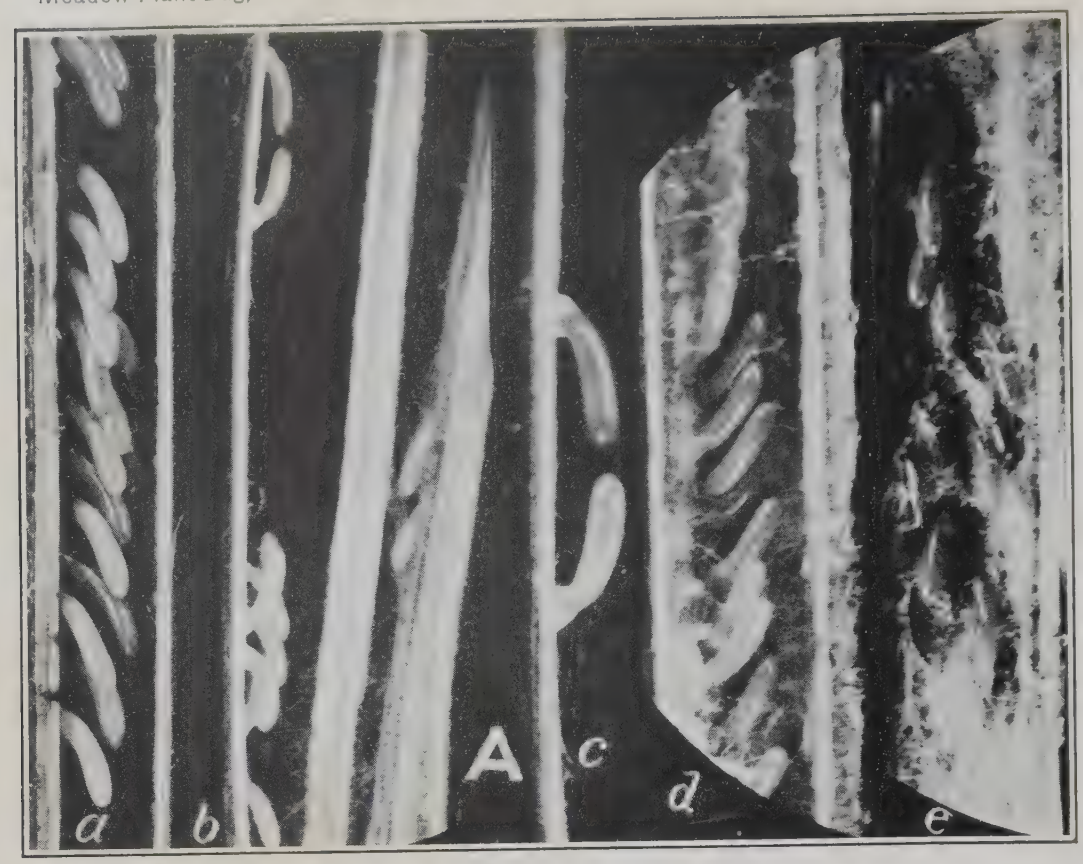
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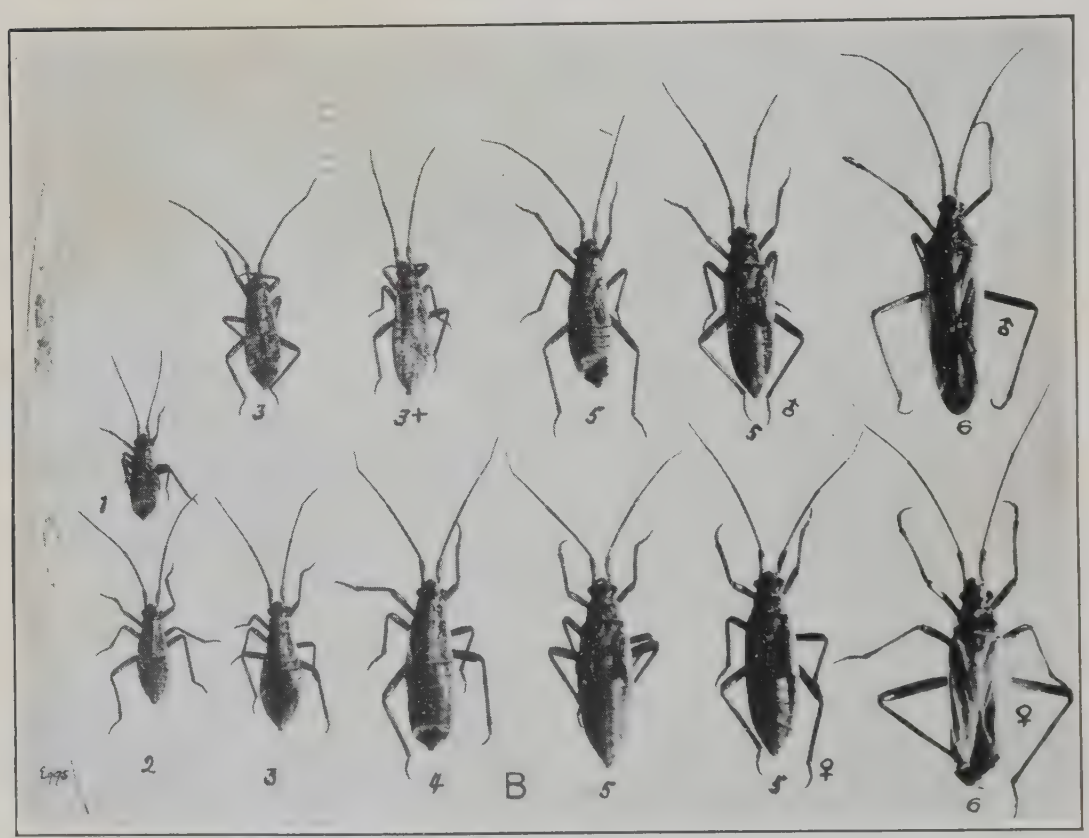
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3
3+
4
B
5
5♂
5♀
6♂
6♀

PLATE 12

Miris dolabratus:

A.—*a*, Eggs in grass stem, all hanging in one direction; *b*, eggs in grass stem placed in opposite directions; *c*, same as upper part of *b*, more enlarged; *d* and *e*, eggs in clover stems. Photographed by Mr. Hammond.

B.—Eggs at left; *1*, first instar; *2*, second instar; *3*, third instar; *3+*, third instar more mature; *4*, fourth instar; *5*, fifth instar; *6*, adult male above, female below. Photographed by Mr. C. J. Drake.

ANGULAR-LEAFSPOT OF CUCUMBER: DISSEMINATION, OVERWINTERING, AND CONTROL

COOPERATIVE INVESTIGATIONS BETWEEN THE UNIVERSITY OF WISCONSIN AND THE BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

The bacterial nature and the symptoms of the angular-leafspot of cucumber (*Cucumis sativus*) have been clearly described by Smith and Bryan (*15*).[2] Prior to this paper the disease had been reported in this country by Burger (*1–4*) and from Europe by Traverso (*16*) and Potebnia (*12*). The two latter writers accepted Burger's statement that the organism which caused the spots on the leaves was also responsible for a serious rotting of the fruit. The inoculation studies made by Smith and Bryan (*15*) showed that the bacterium which caused the leaf-spotting was unable to produce a soft-rotting of the fruit. Extensive tests by the writer have confirmed their finding in this regard.

The bacterial cause of the disease was determined independently by the writer in the summer of 1915, as set forth in a preliminary note (*5*). The morphological and physiological studies which were subsequently made of a strain of the causal organism isolated from a Wisconsin specimen gave results essentially in agreement with those reported by Smith and Bryan (*15*). The name given to the organism by these writers is "*Bacterium lachrymans*." According to Migula's system of classification it would be called "*Pseudomonas lachrymans*."

The damage caused by the angular-leafspot can not be accurately estimated. It varies greatly with differing weather conditions, but enough weather favorable for the disease prevails each year to make the injury of considerable importance (Pl. 13, A). The writer's ideas as to the destructiveness are based mainly on his field experience in Wisconsin and adjoining States during three summers, together with more limited observations in Virginia and southern California.

It is when the disease appears in a field early in the summer that the greatest damage results, as would naturally be expected. Young plants

[1] The writer wishes to express his appreciation to Dr. L. R. Jones, of the Wisconsin Experiment Station, for helpful interest and advice in the prosecution of the work and to thank Mr. W. W. Gilbert and Dr. M. W. Gardner, of the Bureau of Plant Industry, for helpful suggestions and cooperation.

[2] Reference is made by number (italic) to "Literature cited," p. 220.

are often so severely attacked that stunting results (Pl. 13, B). A few scattered plants in a field or nearly all may be so affected, depending chiefly on the meteorological conditions. Some observations on the extent of the injury by angular-leafspot in a representative locality may here be noted to give a more definite idea of the damage which it causes. At Ripon, Wis., in the summer of 1914, sixteen cucumber fields were under observation. The disease appeared in seven of these while the plants were in the seedling stage, and by the middle of the season, August 11, it had resulted in the severe spotting of approximately 25 per cent of the leaves. On August 11 the disease was also present in three of the nine other fields, but in these it had been introduced only a short time and had not yet become generally distributed. A survey of the same locality on August 15, 1915, indicates how widespread the disease may become, especially when the fields are close together. On that date 28 out of 35 fields visited were found to be infested. A later visit revealed even further distribution.

The losses in the regions where cucumbers are grown for pickling purposes result mainly from the decrease in yields due to the destruction of leaf surface, but it seems quite probable that in other sections, as has been pointed out by Burger (*1*, *2*) for Florida, where cucumbers are grown for "slicing" purposes, and so must be shipped to distant markets or kept in storage for considerable lengths of time, an additional loss may come from the secondary soft-rotting of the fruit. Limited observations by the writer indicate that in California the soft-rotting of the fruit as an indirect result of the angular-leafspot may cause some loss. The bacterium causing the leafspot does not directly cause the fruitrot, but through the wounds which it makes on the fruit softrot organisms are frequently able to gain entrance.

The widespread distribution of angular-leafspot and its frequent occurrence give it a place among the major diseases of the cucumber. The aggregate loss which it entails probably exceeds that caused by some of the other diseases which are more destructive in limited areas. In America this disease has been reported from Florida by Burger (*1–4*), and from Connecticut, Indiana, Michigan, New York, and Wisconsin and the Canadian Provinces of Ontario and Quebec by Smith and Bryan (*15*). To this list of regions where it is known to occur may now be added California, Colorado, Illinois, Iowa, Minnesota, and Virginia. That it is probably widely distributed in Europe is indicated by the fact that Traverso (*16*) reported it from Italy, and Potebnia (*12*) recorded its occurrence in Russia. The wide distribution of the disease is a fact that should be expected in view of the evidence to be presented that the causal bacteria are seed-borne and in view of the general occurrence of the trouble in seed-growing localities.

PRELIMINARY CONSIDERATIONS

The chief purpose of this paper is to present evidence bearing on the phases of the problem which are of direct economic significance. Certain other parts of the work which has been done have yielded results worthy of record; and, since some of these results are pertinent to the questions of dissemination, overwintering, and control, they may appropriately be presented before passing to the consideration of the latter points.

DESICCATION

Many questions in regard to the dissemination and overwintering of the causal organism of angular-leafspot depend on its sensitiveness to desiccation. The organism has been shown by repeated tests to be relatively sensitive to drying on glass. With a 3-mm. platinum loop drops were transferred to carefully cleaned cover glasses from 36-hour cultures in beef bouillon and in cucumber-leaf decoction from the leaf exudate and from a suspension in distilled water of the organisms from freshly invaded tissue. None of these showed viable organisms after four days' drying at room temperature. Smith and Bryan (*15, p. 470*) found that the organisms from a young bouillon culture when dried on glass were viable after 21 days. The variance in these results may possibly be due to some slight difference in methods, which may have made a difference in the time for which the bacteria were exposed to complete desiccation.

Freshly invaded fruit and leaf tissue dried in diffuse light at room temperature showed viable organisms after 3 and 10 days, but none were alive after 32 days.

Short periods of drying, four to five days, resulted in the death of all organisms on seed which had been disinfected with mercuric chlorid, washed thoroughly, and then wet with a young bouillon culture of *Bacterium lachrymans*. The fact, however, that the bacteria do survive long periods of desiccation on or in the seed is shown by the evidence to be presented under the discussion of overwintering.

The results of one test on culture media are here pertinent because they show that there are conditions under which the organisms may withstand long periods of drying. On February 2, 1916, six tubes of potato-dextrose agar, in each of which had been suspended approximately 0.5 gm. of powdered calcium carbonate, were slanted and inoculated. The purpose was to see if the life of the cultures might be prolonged by neutralizing with the carbonate the acid resulting from the growth of the organisms. In a dextrose-containing medium the bacteria ordinarily make a rapid growth for a short time and then all die, so that the tubes become sterile, usually within 10 days. The tubes in this test were set away at room conditions and, because of the low relative humidity of the laboratory air, rapidly dried out. Before they dried com-

pletely, which required nearly two months, an abundant growth of the organisms had been made. On November 8, 1916, the dry remains of the agar, carbonate, etc., from three of the tubes were transferred to tubes of bouillon. Growth occurred in all three tubes thus inoculated, and in each case the identity of the organism was established by inoculating cucumber plants. Two of the remaining three tubes were similarly tested on February 9, 1917. Growth resulted in each case, and inoculations again proved that the clouding of the bouillon was due to the angular-leafspot organism. Since all other evidence is opposed to the possibility of the formation of spores, the writer is inclined to explain the survival of some of the bacteria in these tubes by assuming that they were protected from complete desiccation.

THERMAL RELATIONS

The thermal death point of the angular-leafspot bacterium is between 49° and 50° C. Tests were made in 10-cc. portions of beef bouillon in thin-walled test tubes at 46°, 48°, 49°, 50°, 52°, and 55°. Ten minutes' exposure at 46° must have killed a large proportion of the organisms, because growth in tubes so exposed was much slower in appearing than in the unheated controls. In each test some but not all of the tubes exposed at 49° C. showed no growth. In none of the tests did growth occur in tubes exposed at 50° or temperatures above that point.

An interesting contrast between the relation of temperature to angular-leafspot and its relation to the bacterial-wilt of cucumber was brought out at Madison, Wis., in 1916. The maximum temperature as recorded by the United States Weather Bureau there averaged 36.7° C. (98° F.) for the five days July 26 to 30, inclusive. The highest temperature at the Weather Bureau Observatory was 38.3° C. (101° F.), but in direct sunlight and near the ground undoubtedly the temperature was higher. This unusually hot weather did not appreciably check the development of angular-leafspot, which reached its maximum development within about 10 days thereafter, but it practically stamped out the bacterial-wilt. Smith (*14, p. 209*) accounts for the bacterial-wilt having been found only in cool climates on the basis of the low thermal death point, 43° C., of the causal organism.

The relation of temperature to growth in artificial media has been found to agree with the report of Smith and Bryan (*15, p. 470*), and so need not be given in detail.

The sensitiveness of the bacteria to freezing was tested by exposing them in different media in glass test tubes outside a north window during a period of low temperatures in the winter of 1916–17. Dilute suspensions of the bacteria in distilled water, freshly-inoculated tubes of beef bouillon, beef bouillon with approximately 2 per cent of sodium chlorid, and 24-hour agar slope cultures were exposed. During the first 9 days of the

exposure the highest temperature was 0° C. (32° F.), the lowest −25.5° C. (−14° F.), and the average daily mean was −15.5° C. (4.1° F.). One tube of each medium was taken in after 24 hours and longer periods and thawed slowly in cold water. In the salt bouillon all the bacteria were dead after 24 hours. In bouillon without salt all were dead after 60 hours. No test was made of the suspension in distilled water after the 60-hour interval, but no colonies developed in plates poured from one of the other tubes melted after 4 days. On the agar some of the organisms were alive after 6 days, but after 17 days all were dead. The sensitiveness to freezing was undoubtedly increased by the sodium chlorid in the bouillon.

Smith and Bryan (*15, p. 471*) reported freezing the organisms for 15 minutes in bouillon by means of salt and pounded ice. That exposure resulted in the death of nine-tenths of the bacteria.

SENSITIVENESS TO GERMICIDES

Tests of the sensitiveness of the organisms to formaldehyde, copper sulphate, and mercuric chlorid were made. The dilutions of formaldehyde were made up by volume from the 40 per cent formaldehyde solution known commercially as formalin. The copper-sulphate and mercuric-chlorid solutions were made up 1 to 1,000 by weight and the desired dilutions made from these. Exposures were made in all cases by transferring a 3-mm. loop of a young bouillon culture to 10-cc. portions of the dilutions in vials floated on a water bath at 25° C. Tubes of melted agar were inoculated in duplicate or triplicate by a 3-mm. loop transfer from each vial after an exposure of 10 minutes.

The test with formaldehyde resulted in the death of all organisms exposed to a dilution of 1 to 10,000, of nearly all in the 1 to 100,000 dilution, and of apparently none in the 1 to 500,000 dilution. The tests with copper sulphate and mercuric chlorid were repeated twice. With the copper sulphate the results did not agree throughout, but in all cases all organisms were killed or prevented from developing by the 1 to 100,000 dilution. There were no colonies, or a strikingly smaller number than from the controls, in the plates poured from the 1 to 500,000 dilution. All organisms were killed by exposure to dilutions of mercuric chlorid of 1 to 1,000,000.

The sensitiveness of the organism to copper sulphate was tested by Smith and Bryan (*15, p. 474*). Their results show a slightly less marked sensitiveness to this chemical than was found in the tests made by the writer. The temperature at which their exposures were made was not stated.

PLANTS ATTACKED

Little attention had been previously given to the question of the host range of the disease or to the question of variations in susceptibility or resistance to the disease in the case of the different types of cucumbers.

Because of the bearing which these questions might have on distribution, overwintering, and control, 12 horticultural varieties of cucumbers and a large number of other cucurbits were tested as to susceptibility to angular-leafspot. The varieties of field cucumbers and the other species and varieties of cucurbits which are listed in the following table were grown in a cucumber field thoroughly infested with angular-leafspot, where they were under the most favorable conditions for infection. The varieties of forcing cucumbers were tested by inoculation in the greenhouse.

Plants exposed to angular-leafspot infection

PLANTS ATTACKED	PLANTS NOT ATTACKED
1. Cucumber (*Cucumis sativus*), 12 horticultural varieties: Davis Perfect. Chicago Pickling. Boston Forcing. Early Russian. Giant Pera. Japanese Climbing. Heinz Muscatine. Lemon. Thorburn's Everbearing. Rollison's Telegraph. Vaughan's Prolific Forcing. White Spine Klondyke.	1. Balsam-apple (*Momordica balsamina*). 2. Balsam-pear (*Momordica charantia*). 3. Squirting cucumber (*Momordica elaterium*). 4. Pomegranate melon (*Cucumis melo* var. *dudaim*). 5. *Cucumis grossulariaeformis*. 6. Muskmelon (*Cucumis melo*), 11 varieties.[a] 7. Snake melon (*Cucumis melo* var. *flexuosus*). 8. Wild cucumber (*Echinocystis lobata*).[b] 9. Watermelon (*Citrullus vulgaris*), 2 varieties.[a] 10. Citron (*Citrullus vulgaris*). 11. Japanese crookneck squash (*Cucurbita moschata*). 12. Hubbard squash (*Cucurbita maxima*).[a] 13. Turban squash (*Cucurbita maxima*). 14. Summer squash (*Cucurbita pepo* var. *condensa*). 15. Pumpkin (*Cucurbita pepo*).[a] 16. Gourd (*Cucurbita pepo* var. *ovifera*). 17. *Trichosanthes colubrina*.
2. West Indian gherkin (*Cucumis anguria*).	
3. Mandera gourd (*Cucumis acutangulis*).	
4. Hedgehog gourd (*Cucumis dipsaceus*).	
5. Calabash gourd (*Lagenaria vulgaris*).	
6. *Bryanopsis laciniosa*.	

In the case of the plants other than cucumber attacked the causal organism was isolated from diseased spots and identified by inoculating cucumbers. Stained sections from paraffin-embedded material showed bacteria within the tissue of the leafspots in all cases except that of the West Indian gherkin, which was unquestionably subject to the disease.

STOMATAL MOVEMENT AND INFECTION

The fact that leaf infection took place through the stomata was reported by Smith and Bryan (*15, p. 469*), but they gave no discussion of the conditions necessary for infection. Practically all of the earlier inoculations made by the writer were performed in the evening, after dark,

a Greenhouse inoculations also gave negative results.

b The wild cucumber plants were not in the experimental plots, but grew near by and were artificially inoculated.

because the sensitiveness of the organism to sunlight was known and because moisture, such as dew on the leaves, was thought to be the most important factor in infection. A few infections were nearly always obtained in this way, but the number was consistently smaller than occurred on leaves naturally infected. A suggestion that the factors limiting the number of leaf infections were in some way involved with the time of inoculation was obtained when, from a series of inoculations made in the field at intervals of 2 and 4 hours during the day and night of a 24-hour period, more abundant infections resulted from the inoculations made during the day. Evidence that infection occurs more abundantly when inoculations are made during the day was confirmed by other tests.

The idea that during the process of photosynthesis enough oxygen was given off through the stomata to exert a chemotactic action on the causal bacteria was first conceived as a possible explanation of the different results from night and day inoculations. The hypothesis was abandoned after experimental tests. Plants which were kept in darkness for 24 hours before and after inoculation became infected to about the same extent as the controls.

The idea that stomatal movement might be a factor was next hit upon. Pool and McKay (*11*) found that there was a relation between stomatal movement in sugar-beet leaves and infection by *Cercospora beticola*. This fact suggested that in the case of the disease under consideration a similar relation might hold true. To study the behavior of the stomata the method described by Lloyd (*10*) of direct visual observation of the stomata *in situ* was utilized. It was found that the stomata on the lower surfaces of the leaves were generally open during the day and closed at night. The movement of the stomata on the upper surfaces was not always the same as those on the undersides, but this fact is of no special significance here. It was then found by repeated tests that inoculations on the under surfaces made in the morning, when the stomata were observed to be open, gave much more abundant infections than did similar inoculations made at night, when the stomata were seen to be closed (Pl. 14). The following table gives a comparison of the number of infections from night and day inoculations. The two plants used were of the same age, each having seven leaves at time of inoculation. Leaf 7 is the youngest leaf of each. They were treated similarly except for time of inoculation.

Time inoculated.	Leaf 3.	Leaf 4.	Leaf 5.	Leaf 6.	Leaf 7.
7.30 p. m.	0	0	5	48	45
10.00 a. m.	16	43	49	97	50

On the plant inoculated in the evening the youngest leaves, No. 6 and 7, showed many more infections than did the older leaves, and this has been repeatedly found in other inoculations. Why this difference in in-

fection of leaves of different ages occurs is a matter of conjecture, but it is thought to be associated with the fact that younger tissues are more susceptible. Probably the relatively small number of organisms which retain their motility are able, when the stomata open, to establish themselves in the younger leaves, but are not able to gain a foothold in the older tissues.

The closure of the stomata may mechanically exclude the bacteria or may interfere with stimuli which attract them into the interior of the leaves. No attempt has been made to determine this point, but the first theory seems to the writer the more plausible.

FRUIT INFECTION

Fruit infection occurs naturally without wounds. Stomatal infection (fig. 1) has been demonstrated in fruit artificially infected without wounding. Burger's (*4*) description of the effect on the fruit is accurate in part, but the softrot which he emphasizes results from organisms other than the species causing the small, circular, localized spots on the fruit, characteristic of angular-leafspot infections. The circular spots are at first water-soaked in appearance. Later their centers become whitened, owing to a cracking and drying out of the tissues (Pl. 16, B). In fruit, as well as leaf tissue, the bacteria have been seen only in the intercellular spaces.

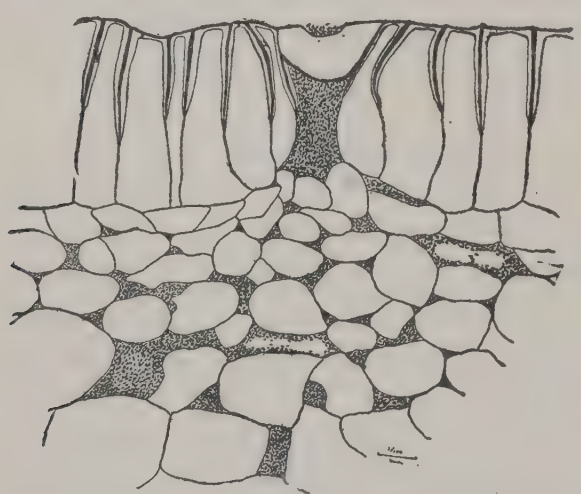

FIG. 1.—Cross section of epidermal portion of cucumber fruit fixed eight days after inoculation with *Bacterium lachrymans*, showing presence of bacteria in stoma and tissues below.

DISSEMINATION

The means by which the disease is spread have been given a good deal of attention because of the possible bearing which these might have on remedial measures. Some of the observations and experiments may throw light on other and similar bacterial diseases.

BY RAIN AND WIND

That the important relation of rainy weather to the progress of angular-leafspot, a factor previously observed, was principally in the dissemination of the causal organisms was made clear in the summer of 1916. Healthy potted plants which had been placed outside of the greenhouse and at a distance of 4 feet from infected plants became diseased after a

rainy period. Experimentally infection was secured by placing recently infected leaves on the ground beneath healthy plants on a day when there were frequent showers. In the fields at Madison newly infected spots appeared in abundance within five or six days after heavy rains, especially the rains of July 19 and August 3–5. Rain must fall at relatively frequent intervals to be effective in spreading the disease. Prolonged rainless periods check the development of the disease to a great degree, especially if accompanied by high temperatures (Pl. 15, C).

The importance of rain in the development of the disease was clearly shown at Ripon, Wis., in 1914. Owing to favorable rainy weather early in the season angular-leafspot spread throughout certain fields. Two of the infected fields at Ripon and one in a neighboring locality, which were visited on August 11 and 12, presented a striking appearance.

The vines were so grown together as to nearly cover the ground, but the centers of the rows were clearly marked by the old, angular-spotted leaves in contrast with the healthy green of the later growth which had developed after the last heavy rain.

Further evidence regarding the importance of rain in relation to the development of the disease was furnished by a comparison of conditions at Madison and Ripon, Wis., in 1916. The striking difference in the amount and distribution of rainfall for the two places during the month of July can be seen in Table I.

TABLE I.—*Dates and amounts of rainfall at Madison and Ripon, Wis., in July, 1916*

Day of month.	Precipitation (inches).	
	Madison.	Ripon.
1	0.00	0.13
12	.33	.11
16	.90	.00
19	1.21	.00
20	.19	.00
22	.03	.00
26	.00	.02
Total	2.66	.26

The time of planting and the earlier weather conditions were similar, and so it is highly probable that the disease appeared in both localities at about the same time, noted first at Madison on July 3. At the end of the month the disease was widespread and affecting leaves of all ages in the Madison fields, while at Ripon only the older leaves at the hill centers showed the angular spots.

The relation of wind to dissemination by rain spattering has not been studied experimentally, but the comparison of the way the disease spread in differently situated fields throws some light on the question.

One field, which we may call field A (fig. 2), was on the southeast slope of a hill and surrounded by trees so that it was well protected from wind, especially northwest wind. Field B was on the west slope of another hill and freely exposed to wind. The original centers of angular-leafspot in B were on the north side near the top. Thunder showers on July 12, 16, and 19 were accompanied by high winds from the north and west. On July 29 it was noted that in field A the infested areas were strikingly more delimited than those in field B. The difference could be explained only as a result of the difference in exposure to winds.

Another field was situated on a freely exposed west slope, and its rows ran with the hill, east to west. No notes on disease distribution there were taken until August 4. On that date there was a center of abundant

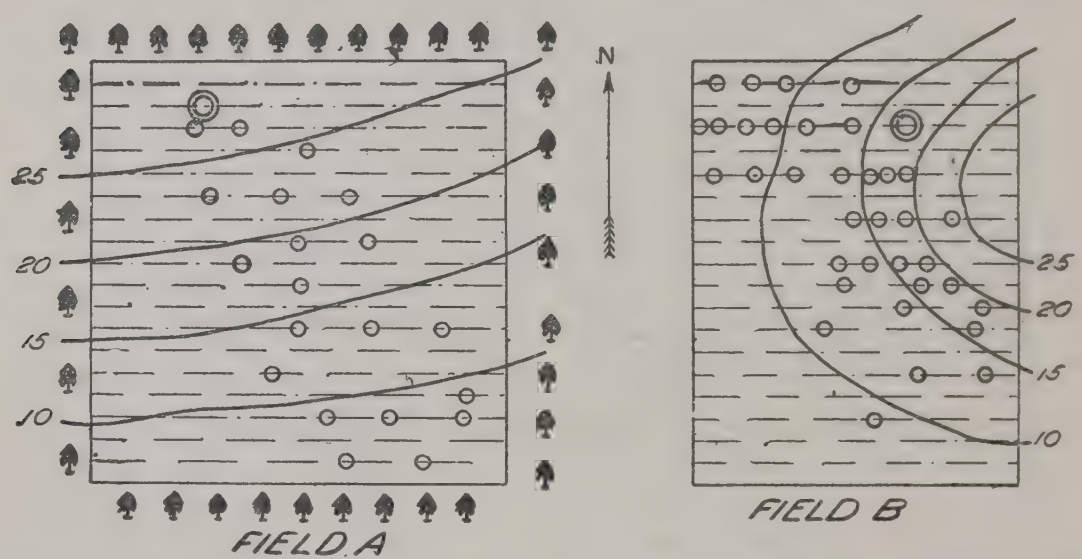

FIG. 2.—Diagrams of cucumber fields to show relation of wind and drainage water to angular-leafspot dissemination. Double circles indicate the location of original centers of infection prior to storms. Small circles represent secondary infections due to storms. In field A drainage water only was an important factor because of protection from wind while in the freely exposed field B wind also played a part. Contour lines indicate elevation above level of Lake Mendota, Madison, Wis. Broken lines show the direction of the rows. For full explanation see text.

infection in the seventh row from the north side and scattered infections in all of the 16 rows south of it. North of this badly infested area the adjacent row, the sixth, showed a very small number of infections, and the 5 others were entirely free from the disease. Obviously the northwest winds had played an important part here also in spreading the disease. Faulwetter (7) has shown that wind in connection with rain is an important factor in the spread of a similar bacterial disease, the angular-leafspot of cotton.

The fact that the thundershowers mentioned as resulting in a marked spread of angular-leafspot occurred during the daytime supports the inference which may be drawn from the facts regarding the relation of stomatal movement to infection, that rains which occur in the daytime are more effective in the spread of the disease than are those occurring at night.

BY DRAINAGE WATER

Evidence concerning the distribution of the causal organism by drainage water during rains was afforded by comparing developments in fields A and B which were mentioned in the preceding section. The rows in field A ran across the hillside, while in B they followed the direction of the slope (fig. 2). After the rains of July 12, 16, and 19 the disease appeared in field B throughout the length of the rows in which it had been noted earlier and in plots below them where disinfected seed had been planted. In field A, however, the spread of the disease was not mainly along the rows but rather crossed the rows, following the path of the drainage water. The supposition is that the organisms were carried by the drainage water and from it were spattered by the rain to the healthy plants. Dissemination by drainage water has been noted before with fungus diseases—for example, cabbage-yellows by Jones and Gilman (*9*)—but, so far as is known to the writer, no evidence has before been published in regard to its significance in the case of a bacterial disease.

Attempts to prove that drainage water carried the causal organisms were made on two occasions late in the summer. Samples of drainage water caught during rains at the lower edges of infested fields were taken to the greenhouse and sprayed on healthy plants. The negative results are not surprising in view of the fact that few new infections developed in the fields where the water was caught, and that negative results from attempts to isolate the bacterium from beetles from these same fields also indicated that a large proportion of the bacteria had been killed as a result of the long, preceding period of dry weather.

BY PICKERS

The spread of diseases due to fungi has been attributed to pickers—for example, bean anthracnose, by Whetzel (*17*)—but, so far as the writer is aware, no such fact has been demonstrated for a bacterial disease. Experiments in the case of the cucumber angular-leafspot have shown that the disease may be spread by pickers if picking is done when the exudate is present on the infected leaves. On August 8 and 9, 1916, the matter was tested as follows: At 5.30, 7.30, and 8 o'clock on the morning of the first day and at 8 a. m. on the second day two or three leaves in each case were inoculated by rubbing with the hands (as is done by pickers) after having first rubbed them through the exudate on diseased leaves. In all four cases inoculated leaves became infected (Pl. 15, B), while the uninoculated controls remained healthy.

Picking is, of course, frequently done early in the morning and on rainy days when the leaves are wet and the bacterial exudate is abundant. Numerous observations show that the spread of the disease in the way described in the preceding paragraph often results. The most obvious

of the cases that have come under the writer's notice seems worth mentioning in detail. At Princeton, Wis., a patch of cucumbers of seven rows was visited on August 12, 1916. In the middle of the third row, counting from the north side, there was a circular area of diseased leaves, badly shattered by the rain of August 10. West of this area of shattered leaves no new infections were evident. East of this area, however, there were numerous recent infections, and the number of these varied nearly inversely as the distance from the original center. The location of diseased leaves and the position of the spots on them corresponded to observations on dissemination by pickers made in other places. When passing the patch later in the day the owner was seen starting to pick on the west end of the first row, so that when he would come to pick the third row he would be working eastward (fig. 3). Evidently he must have followed the same course when the bacterial exudate was abundant enough to thoroughly contaminate his hands.

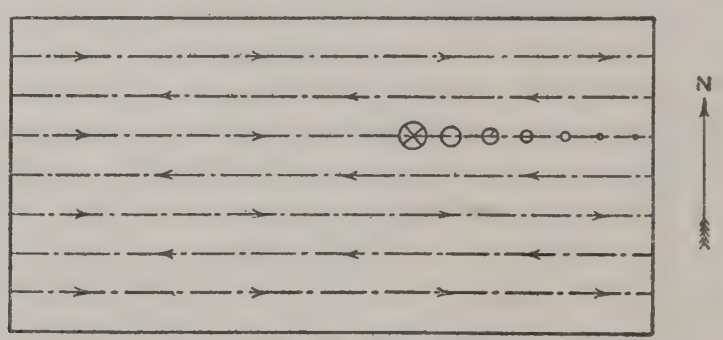

FIG. 3.—Diagram of cucumber field to illustrate picker dissemination of angular-leafspot. ⊗=original center, ○=new infections, arrows indicate direction the picker worked.

BY INSECTS

Cucumber beetles (*Diabrotica vittata* Fab. and *D. duodecimpunctata* L.) have repeatedly been seen crawling over infected leaves and flying about the fields when the bacterial exudate was plentiful, as early as 5.30 a. m. Platings from these insects were made in only a few cases. That some of them would crawl through exudate and become contaminated seemed unquestionable. In one instance platings from a water blank, in which had been dropped three 12-spotted beetles, yielded the causal organism.[1] Bees have been observed visiting the plants as early as 7.30 a. m. and have been seen to occasionally brush against exudate-bearing leaves.

The carrying of the causal bacteria from one part of a field to another by insects is no doubt significant, but in view of the other ways in which the local spread is accomplished it is far less important than is the dissemination of the organism by the same agency from diseased to healthy

[1] The organism in this case was not tested as to pathogenesis, but was identified by colony characters on potato-dextrose agar. These typical colonies were more numerous than any other kind, and by transfers (unintentionally delayed until 10 days had passed) it was shown that the organisms were dead, as is true for the angular-leafspot organism with media containing dextrose.

fields. The evidence supporting the latter idea is observational. During 1916 six experimental fields were grown near Madison in the same vicinity with four privately owned commercial fields. The distances between the commercial and the experimental fields varied from about 30 rods to ½ mile. Angular-leafspot appeared in all six of the experimental plots early in July. It appeared in only three of the others and in these not until nearly the middle of August. Prior to this, in the forepart of the month, there was a period in which there was an abundance of bacterial exudate in the infested fields and when insects, especially the beetles, were very active. It is of interest here to note that in no case did the original center of infection in a private field develop at the edge, but rather in the interior of each of the three patches. It may be stated with confidence that during the time in question no one except Dr. M. W. Gardner and the writer visited both the experimental and commercial fields, and as these visits were made when the leaves were dry there seems little probability of the organisms having been transported by us. Comparable developments were observed at Ripon, Princeton, and Pittsville, Wis.

Closely correlated with picker dissemination and the probability of spread by insects is the relation of atmospheric humidity to the disease. Under conditions of high relative humidity, such as frequently prevail on summer nights, the invaded areas of the leaves take on clear-cut angular shapes and the bacterial exudate becomes abundant. (See Pl. 13, A). Such nights were those of August 8 and 9, 1916. The relative humidity, as recorded by a Friez hygrograph, varied on those nights from 74 and 80 per cent, respectively, at 7 p. m. to 90 per cent, where it continued until 6 a. m. Observations in the early mornings showed abundant signs, as described, that conditions for the progress of the disease had been most favorable. In steam-heated greenhouses, with the relative humidity varying from 45 to 60 per cent, the disease develops poorly or not at all.

OVERWINTERING

The several ways in which the causal organisms of other bacterial diseases have been thought to pass the winter have been kept in mind in searching for evidence as to how the angular-leafspot bacterium overwinters.

SOIL

The sensitiveness of the organisms to freezing, as elsewhere recorded, renders doubtful the possibility of their living over the winter in the soil or in the débris of diseased vines in northern climates. A limited amount of work on this question indicates that the bacteria do not live for long periods in the soil. The question can not, however, be definitely settled until further study of it has been made.

INSECTS

The hypothesis that the bacteria may overwinter in or on the bodies of insects is here mentioned because it might seem plausible in view of the theory advanced with good evidence by Rand and Enlows (*13*) to account for the overwintering of the organism causing the wilt of cucurbits. No dependent relationship, such as has been found to exist between the wilt and cucumber beetles, has been observed in the case of angular-leafspot. Field observations in 1916 furnish some good negative evidence relative to the insect-overwintering theory. In one vicinity near Madison six cucumber fields on "new" land planted with seed from one source became diseased early, while four fields (planted with seed of a different source) on or very near land which had previously been planted with cucumbers did not develop the disease early. The early brood of beetles was fully as abundant on the four latter fields as on those six that became diseased early in the season.

SEED

The observations which formed the preliminary basis for the seed-overwintering theory have been printed before (*6*), but for the sake of bringing together all the pertinent evidence may be here repeated. In June, 1915, angular-leafspot was observed in abundance in a field south of Portsmouth, Va. The plants were developing their fifth and sixth leaves at the time. The field was on newly cleared land, surrounded by woods and at least 3 or 4 miles from the nearest cucumber patch. The evidence pointed strongly to the introduction of the organisms with the seed.

The developments in the fields near Madison in 1916 gave further evidence that the organisms are introduced with the seed. The six experimental fields previously mentioned were all on land which had not been planted to cucumbers for at least three years. Angular-leafspot appeared on seedlings in all six of these fields, and in three of them it was noted on the cotyledons. In the case of the four commercial fields near by which were planted with seed from another source the disease did not appear at all in one and not until late in the season in the other three. This evidence so strongly indicated that the bacteria live over winter on the seed that it seemed worth while to study the matter in the commercial seed fields. Accordingly the writer visited a large seed-producing center in Iowa and Dr. M. W. Gardner, because of his interest in the question in relation to cucumber anthracnose, visited a seed farm in Ohio. In one of the seed fields in Iowa the disease was widespread and, according to a hasty estimate, 25 per cent of the fruits were attacked. Dr. Gardner found spots on the fruits in the Ohio fields which he was reasonably sure were due to the angular-leafspot organism.

Since the fruit invasions are local and shallow, it is evident that the seed rarely, if ever, becomes attacked naturally. A study of the way the seed is thrashed, however, sheds further light on the way in which the seed may become contaminated.

The thrashing process practiced on the farms visited is probably in general use. It is begun by shoveling the whole fruits into a grinding machine which chops them up and allows the larger parts of the fruit pulp to be carried off on a rotating screen. The seed, the juice, and the smaller pieces of pulp fall through the screen and are drained into containers. This much of the process would doubtless afford ample opportunity for the organisms to reach the seed. The next step, however, probably increases the chances for the seed to become contaminated. The seed with the pulp and juice is left in the barrels with frequent stirring for a period of time varying usually from one to three days. The angular-leafspot organism doubtless multiplies rapidly in this well aerated mixture of juice and pulp unless conditions become unfavorable owing to the by-products of other organisms. After the material containing the seed has stood in the barrels for the time mentioned, it is poured into other containers and the seed separated out as well as possible by repeated washings with water. Then the seed is dried on shallow trays, at first in the sunlight and later indoors. The process of thrashing includes no step which would be likely to kill all the bacteria.

Seed for further study was sent to Madison from both the Iowa and Ohio farms. The details of some of the experiments performed with this seed and the results are here summarized.

EXPERIMENT OF FEBRUARY 20, 1917.—Sixteen flats of sand were steamed at 7 pounds' pressure for one hour. Then each flat was planted with approximately 150 seeds from Iowa. Before touching the seed the hands were rinsed in 70 per cent alcohol as a precautionary measure. After the flats were planted they were wet down with water that had been boiled (cooled), and boiled water was used in all subsequent watering. None of the resulting seedlings were diseased.

EXPERIMENT OF MARCH 3, 1917.[1]—Fourteen flats of sand were steamed as before. The hands were disinfected with mercuric chlorid and alcohol. The trowel was treated with hot water. Twelve flats were planted with seed from the lot from Ohio, about 100 seeds to each flat. One flat was planted with seed from the 1915 supply which had been treated with 1 per cent formaldehyde for 20 minutes and another with seed from the same lot which had been treated with 1 to 1,000 mercuric chlorid for five minutes—these two for controls. The flats were covered with sterilized wire screen to protect them from mice and rats. The flats were watered with water (cooled) which had been boiled.

On March 19 the writer found four seedlings in one of the flats showing typical signs of angular-leafspot as they had been observed on seedlings artifically infected by planting inoculated seed and on naturally infected seedlings in the field (Pl. 15, A). The attacked seedlings were in two separated places—two affected seedlings next to each other in each case—and apparently one seedling had been infected from its neighbor in each instance. From a seedling from each of the two places the organism was isolated and used in pure culture inoculations to reproduce the disease. Stained sections

[1]Performed by Dr. M. W. Gardner in connection with his work on cucumber anthracnose.

of one of the spots on one of the cotyledons showed bacteria in the intercellular spaces. Two of the seedlings were preserved as herbarium specimens. On March 27 another infected seedling was noted in another flat. The organism was isolated and identified by inoculation as before.

EXPERIMENT OF MARCH 27, 1917.[1]—Fourteen flats of sand and four of heavily composted garden soil were steamed for one hour at 7 pounds' pressure. All but two were planted with the seed from Ohio. These two planted with seed treated in 1916 with mercuric chlorid and untreated seed from the 1916 supply, respectively. Precautionary measures taken as before. On April 4 a typically infected seedling was noted in one of the sand flats planted with the Ohio seed and on April 7 a well-advanced stage of the disease was discovered on a seedling in another sand flat of the Ohio seed. There was no doubt as to the cause of the lesions from the characteristic signs—viz, water-soaked tissue and white exudate residue. Platings from each of these seedlings gave an abundance of the typical colonies.

The results of these experiments and the fact that in Dr. Gardner's later tests of the Ohio seed in sterile damp chambers one seedling in each of two damp chambers developed the typical signs of the disease prove that the angular-leafspot organisms may live for at least seven months on the seed. There seems no reason to doubt but that they can survive for two months longer and infect the seedlings as field observations have indicated.

The use of seed as badly contaminated as the lot from Ohio was found to be, would have resulted in the early development of angular-leafspot in as large a proportion of the fields as occurred in 1916 in Wisconsin. From the Ohio lot approximately 3,500 seeds were planted with the precautions described. Seven, or a proportion of 1 to 500, of the resulting seedlings developed angular-leafspot. With this proportion or 0.2 per cent and the use of 2 pounds of seed per acre, as is usually practiced, there would be about 72 plants infected from seed-borne organisms to every acre of cucumbers.

As to how the organisms are protected on the seed so as to withstand the long period of desiccation there is no conclusive evidence. It seems most likely to the writer, however, that they get in at the micropylar end of the seed, and so are protected within the seed coat. The fact that the infections of the seedlings nearly always occur on the edge of the cotyledons near the point of attachment to the stem—the part of the cotyledons which is at the micropylar end—indicates that the bacteria are probably harbored beneath the seed coat (Pl. 16, A). It might be argued that, since on germination the attached ends of the cotyledons are the first to emerge, the portion which becomes infected is the first part which is exposed to organisms on the surface of the seed. This explanation, however, seems less probable to the writer than that the organisms are sheltered inside the micropyle. At any rate subsequent work by Gardner and Gilbert (*8*) has shown that the bacteria are so located that they can be killed by chemical treatment of the seed.

[1] Performed in cooperation with Dr. M. W. Gardner.

REMEDIAL MEASURES

The matter of finding some means of controlling angular-leafspot has been kept in mind in all the studies, especially in comparing cucumber varieties as to susceptibility, in observing the ways in which the disease is spread, in testing the sensitiveness of the organism to desiccation, to heat and chemical germicides, and in trying to determine how the bacteria are overwintered.

RESISTANT VARIETIES

Tests made in the field in 1915 and 1916 by growing the horticultural varieties (listed on page 206) where they were exposed to infection yielded no encouraging results. There was no marked difference in susceptibility between the varieties. No instance of individual resistance has been observed in all the fields which have been examined.

SANITATION

The evidence recorded under the section on dissemination by pickers justifies the recommendation that where feasible the picking of fields into which the disease has been introduced be done at times other than in early mornings or on rainy days when the bacterial exudate is abundant. In cases where it is necessary to pick over a partly diseased field under those unfavorable conditions it may be worth while to pick the healthy part of the field first.

The hope for the complete control of the insect pests, particularly the cucumber beetles, seems to be a thing for which there is little basis. The fact, however, as discussed under the consideration of dissemination, that there is good evidence that these insects are instrumental in spreading the disease from one field to another makes more urgent the need of finding better ways of holding them in check.

SPRAYING

Spraying experiments in which Bordeaux mixture (3–6–50) was the principal fungicide used, were under observation in Wisconsin during the summers of 1914, 1915, and 1916. Noticeable checking of the disease resulted each year. Yield results were in all cases so vitiated by factors other than the spraying, especially the mosaic disease, that comparisons of them were of little value. Furthermore, the disease did not develop in the most destructive way on the experimental fields. The data at hand therefore hardly justify a definite statement of the value of spraying for this disease, but, in the opinion of the writer, the practice would not in Wisconsin and neighboring States be generally profitable on a commercial scale. Several reasons have furnishd the basis for this conclusion. Because of the early appearance of the disease, spraying, to be most effective, would have to be started nearly as soon as the plants came up. Because of this need for beginning early and continuing the

spraying at frequent intervals throughout the season, the cost would probably be greater than could be compensated by the resulting increase in yield. Cucumber vines normally grow so rapidly that the intervals between spraying would have to be short in order that a considerable portion of the younger leaves would not be exposed to infection a good deal of the time. The fact, however, that the disease is mainly dependent on rain for dissemination and that long, rainless periods occur at irregular times would make it a hard matter to recommend a spraying schedule which would be economical.

Spraying where profitable because of other considerations has no doubt been of increased value because of the partial protection afforded from angular-leafspot damage.

Burger (*4*) reported beneficial results from spraying for this disease on the basis of a limited amount of spraying in one season. He found a decidedly smaller number of infected fruits in the sprayed than in the unsprayed plots, and reported that the leaves in the sprayed plots were healthier than those in the check rows. It is interesting to note, however, that his recorded yields show that in every case the total yield, including infected and healthy fruits, was greater from the check than from the sprayed plot. This fact may be correlated with the unsettled question of spray injury to cucumber.

The readiness with which angular-leafspot is spread by spattering of rain makes a spraying experiment, in which the check rows are parallel and adjacent to those sprayed, incomparable to the spraying of a whole field. This fact should be borne in mind when further spraying tests are made.

SEED TREATMENT

The evidence indicates strongly that the angular-leafspot organism overwinters principally on the seed. If this be true, the matter of controlling the disease is greatly simplified, especially from the standpoint of the industry of growing cucumbers for pickling. Some of the pickle companies grow their own seed, while others buy seed from seedsmen. All companies, so far as is known to the writer, furnish the seed to the growers with whom they contract to raise the cucumbers. There will be little difficulty, therefore, in getting the seed disinfected before it is distributed to the farmers, after a satisfactory method of treatment has been worked out.

Preliminary tests of treatments with hot water and with chemical disinfectants have been made. Seed has been treated as follows: Soaked in water at 50° and 52° C. for 10 minutes; in formalin (4 per cent) for 5 minutes and 2 minutes; in copper sulphate (1 per cent) 10 minutes and 5 minutes; and in mercuric chlorid (1 : 1,000) for 5 minutes and 2 minutes. These tests were run on such a small scale because of limited greenhouse space for testing germination that conclusions can not be drawn as to

the effectiveness of the treatments in killing the causal organism, but they do indicate that no important injury[1] to the seed from these treatments may be expected. Extensive field tests with treated seed and further field trials of disinfectants with special reference to injury to the seed are under way in Wisconsin, Michigan, and Indiana under the supervision of Mr. W. W. Gilbert and Dr. M. W. Gardner.

SUMMARY

Angular-leafspot of cucumber was first noted in Wisconsin in 1914 and its bacterial nature established in 1915. The disease is the same as that described by Smith and Bryan (*15*) and earlier reported by Burger (*2*), Traverso (*16*), and Potebnia (*12*).

The disease is probably world-wide in its distribution. Under favorable meteorological conditions it does a good deal of damage. Because of its widespread and frequent occurrence it should be ranked among the cucumber diseases of major economic importance.

Leaf infection is stomatal. Inoculations made at different hours showed that infection occurs chiefly during the day rather than the night. This is probably to be explained by the fact that the stomata are open during the day and closed at night.

Fruit infection is stomatal. The disease first appears there as small, localized, circular, water-soaked spots. The centers of the spots later become whitened, so that they are more readily noticed.

Rain is the most important means of dissemination, but pickers and probably insects play a part in this process.

The causal organism is sensitive to desiccation, is readily killed in artificial media by freezing, is killed in liquid media by an exposure for 10 minutes at 50° C., and is readily killed by dilute solutions of formaldehyde, copper sulphate, or mercuric chlorid. The sensitiveness of the organism to these chemicals is increasingly greater in the order mentioned.

There is substantial evidence that the causal bacteria overwinter with the seed.

No marked variation in resistance or susceptibility has been found among horticultural varieties of cucumbers. A few ornamental gourds are attacked by the disease. Attacks are limited to the cucurbits, and in that family no important crop plant other than the cucumber has been found affected.

Sanitary measures, such as precautions in picking and in control of insects, may be helpful. Spraying with Bordeaux mixture checks the disease, but is of doubtful value as a general commercial procedure in regions where spraying would not otherwise be practiced. Seed treatment offers the greatest hope of satisfactory control.

[1] In the subsequent field tests carried on by Gilbert and Gardner, the 4 per cent formalin treatment caused considerable injury to cucumber seedlings, resulting in marked rolling of cotyledons and retardation of growth. The mercuric chlorid treatment (1: 1,000 for five minutes) has proved safe and effective (*8*).

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PLATE 13

A.—Cucumber leaf five days after inoculation with *Bacterium lachrymans*, showing severe infection. The dark, angular spots had a water-soaked appearance. Drops of bacterial exudate may be seen on some of the spots. Photographed by Mr. Fred R. Jones.

B.—Plant a, photographed seven days after inoculation with *Bact. lachrymans* shows considerable stunting as compared with the uninoculated control, plant b. Young plants as severely attacked have often been seen in the field.

A
a
B
b

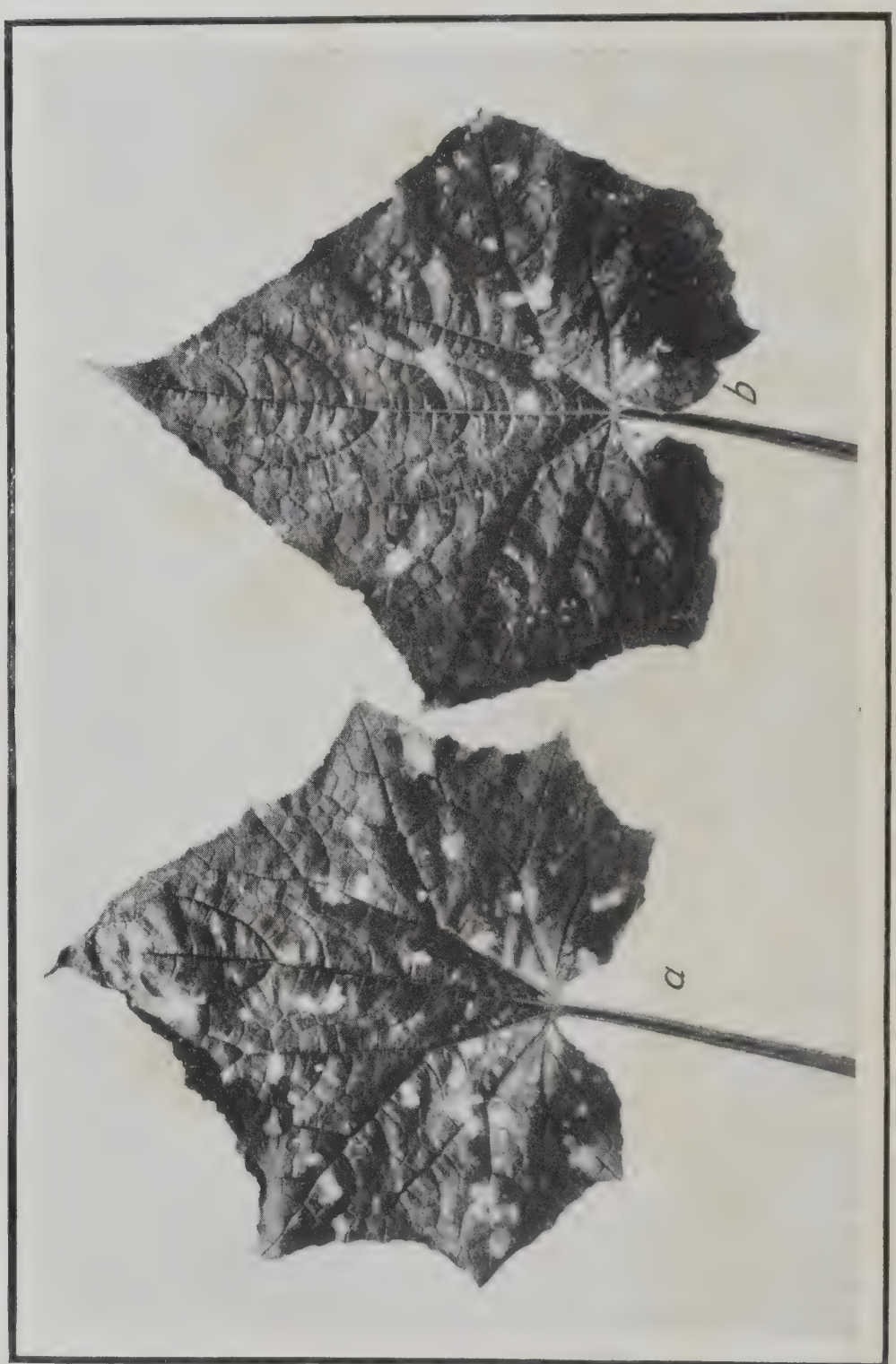

b
a

PLATE 14

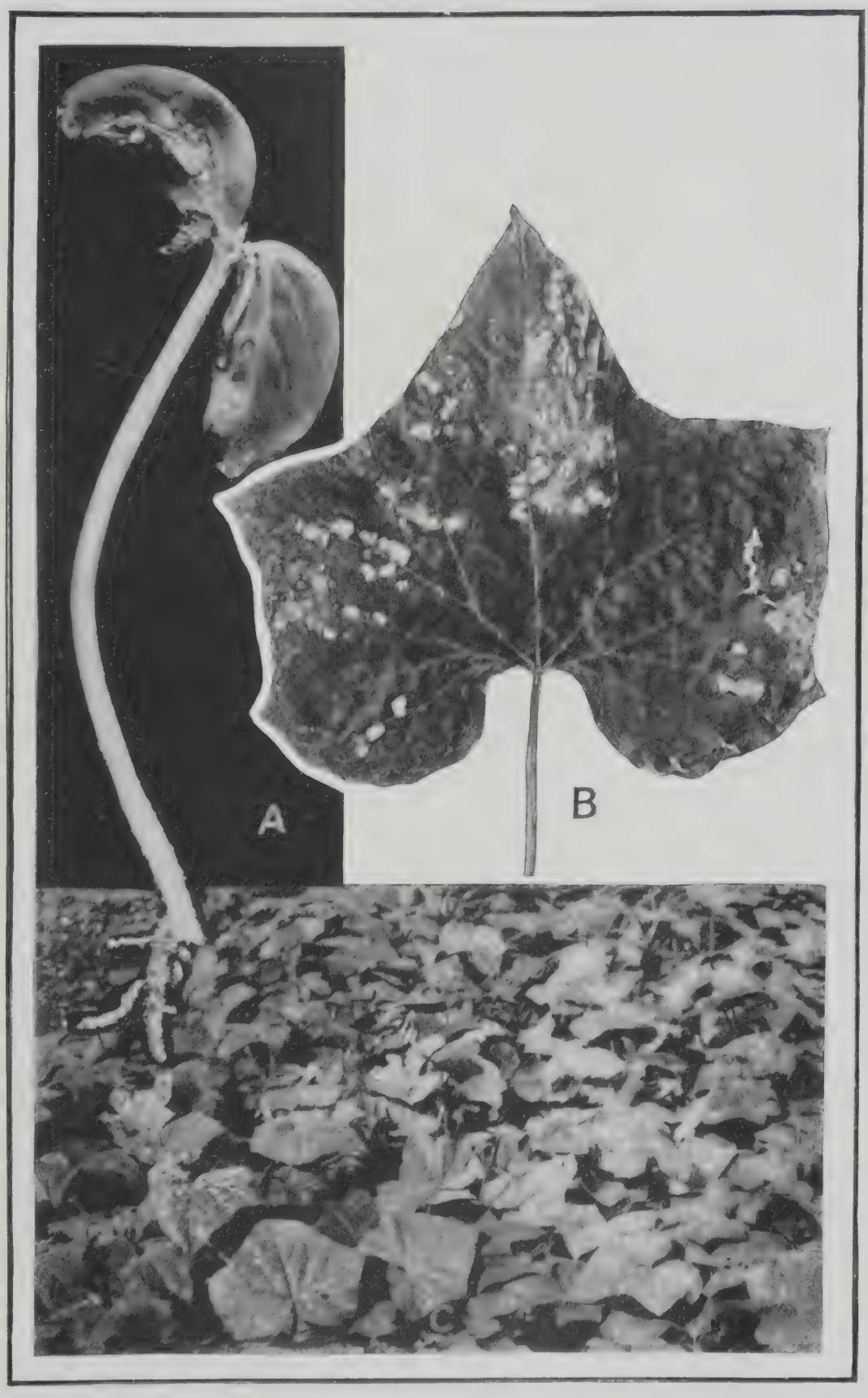
Stomatal movement in relation to infection. The cucumber leaves used in the experiment were of the same age and on similar plants. So far as possible, conditions of inoculation were similar except that leaf a was inoculated with *Bacterium lachrymans* at 9.15 a. m. and leaf b at 6 p. m.

PLATE 15

A.—Overwintering on seed: Natural infections on cotyledons of seedling grown in steamed sand from commercial seed which had been kept in storage for seven months after harvesting. Experiment of March 3, 1917. Enlarged about 1½ times.

B.—Picker dissemination. Infection resulting from inoculation of a cucumber leaf at 7.30 a. m. by rubbing with the hand immediately after touching diseased exudate-bearing leaves.

C.—Dissemination by rain. The older leaves in the center of the row were badly infected during a rainy period. Young leaves on the sides of the row which developed during a rainless period are comparatively free from the disease. Photographed by Mr. W. W. Gilbert.


A
B

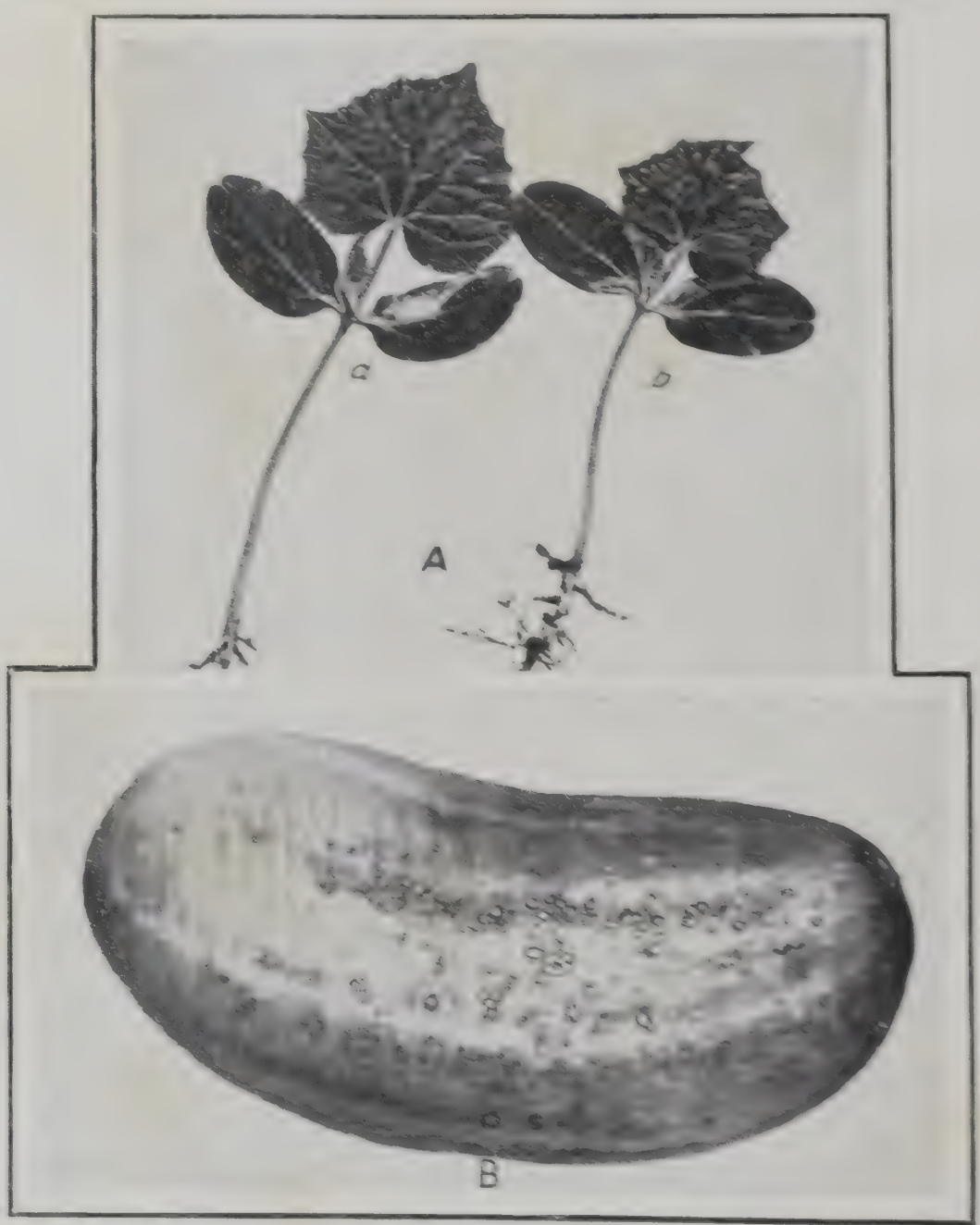

C
D
A
B

PLATE 16

A.—Seedling infection resulting from seed inoculation with *Bacterium lachrymans*. Seeds were wet with a pure culture of the angular-leafspot organism and planted in sterilized soil. Note location of cotyledon infections. Photographed 14 days after planting.

B.—Cucumber fruit showing small, watersoaked, circular spots with white centers resulting from natural infections with angular-leafspot.

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PLASTICITY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS

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COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE[2]

INTRODUCTION

Ever since the discovery of biologic forms of *Puccinia graminis* Pers. by Eriksson (*5*) [3] there has been much speculation as to the degree of fixity of these forms. Eriksson (*6, p. 657*) expresses the opinion that, on account of host and climatic influences, the forms may gradually change. His conception of a biologic form is that it is the result of an adaptational tendency. Magnus (*13, p. 366*) seemed to be of the opinion that biologic forms are the result of association with the particular host plants which they attack. Dietel (*4*) and others held essentially similar views. In general these assumptions have been considered reasonable.

Ward (*22, 23*), as a result of exhaustive investigation of the brown rust of bromes *Puccinia dispersa* Erikss., concluded that biologic forms of this rust could be changed parasitically by association with the proper host plants. He found that, whereas it was often impossible to transfer the rust directly from one species of Bromus to another species, this result could sometimes be accomplished by infecting a third species on which the rust acquired the capability of infecting the normally immune species. Such species he designated bridging species. His opinion appears to have been that taxonomic relationships among the species of Bromus were the determining factor. If, for example, the rust on a given species, A, could not be transferred directly to another species, C, it might be transferred to B, intermediate taxonomically between A and C. The species B then changed the rust sufficiently to enable it to infect C. Having once established itself on C, it was thenceforth capable of

[1] On leave.

[2] Published, with the approval of the Director, as Paper 127 of the Journal series of the Minnesota Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," p. 250–251.

infecting it easily. Freeman (*8*) did similar work and came to the same conclusions. No attempt has yet been made to repeat the work of these investigators, but the writers have observed that some species of Bromus are hosts for most biologic forms of *P. graminis*. *Bromus tectorum*, for instance, can be infected by all the common forms of *P. graminis* in the United States. It is possible that the conditions of experimentation on which the idea of bridging is based were not rigid enough to exclude all possibility of working with a mixture of biologic forms. On the other hand, it is quite possible that the brown rust of bromes with which Ward and Freeman worked was an unstable, easily changed form.

Salmon (*16*) added considerable evidence to the concept of the efficacy of bridging hosts in widening the host range of biologic forms by his experiments with *Erysiphe graminis* DC. on various species of Bromus.

Freeman and Johnson (*9*) applied the principle of bridging hosts to *P. graminis*. They state (*p. 20*) that—

> The barley stem rust enjoys the widest range of any of the biologic forms of the cereal rusts. On the other hand, a transfer of any of the other stem rusts to barley widens the range of that rust. We have here, then, a decided reaction of host upon parasite, enabling the latter to adapt itself to hosts not ordinarily congenial; for instance, W——>B——>O.

Johnson (*11, p. 10*) obtained similar results with timothy rust. He states—

> A small number of experiments to test whether or not the timothy rust can be transferred by means of bridging hosts to various cereals which are not successfully infected directly from timothy were tried, and it was found that by using *Avena sativa* as a bridging host the rust easily transferred to *Hordeum vulgare* (4 times in 10 trials); and by using *Festuca elatior* it transferred to *Hordeum vulgare* (twice in 10 trials) and to *Triticum vulgare* (once in 10 trials); and by using *Dactylis glomerata* it transferred to *Triticum vulgare* (once in 5 trials). By the use of the bridging hosts the rust undoubtedly could be made to transfer to many grasses on which it will not grow when coming directly from timothy, but on which it might continue to grow after such a transfer. That this takes place to some extent in nature is very probable, and these trials, together with recent experiments of a similar nature on the rusts of grains, throw much light on the possible origin of many of the so-called "physiological species" of rust.

Pole Evans (*7*) stated that hybrid wheats could also act as bridging hosts, enabling *P. graminis* to infect the susceptible parent more vigorously and even to attack the highly resistant or almost immune parent. Biffen (*2*), however, obtained no evidence of such remarkable changes.

Arthur (*1, p. 227–228*) cited evidence to show that barberry (*Berberis* spp.) may also act as a bridging host, enabling "racial strains" of *Puccinia poculiformis* (Jacq.) Wettst. (=*P. graminis* Pers.) to increase their range of infection capabilities. Bolley and Pritchard (*3*) and others attributed to barberry a "reinvigorating function" for the rust, although not necessarily a bridging function.

On account of the weight of the above-cited evidence the role of bridging hosts in breaking down biologic-form specialization has been given fairly general credence.

But Eriksson (*5*), Jaczewski (*10*), Freeman and Johnson (*9*) and Stakman (*18*) could detect no clearly appreciable influence of barberry on the parasitism of biologic forms of stemrust. Stakman (*18*), Stakman and Jensen (*19*), and Stakman and Piemeisel (*20*), in rather limited experiments, could not duplicate the results of Freeman and Johnson with *P. graminis* nor those of Johnson with *P. graminis phleipratensis* (=*P. phleipratensis*). Stakman (*18*), however, obtained results indicating that possibly changing the host metabolism by the use of anesthetics and fertilizers might increase the parasitic capabilities of the rust slightly, thus giving some support to the work of previous investigators (*12*, *14*, *15*, *17*) along similar lines.

On account of the undoubted theoretical and practical importance of the problem it seemed desirable to make extensive experiments with a number of forms of rust from different hosts from different regions. Work was therefore begun in the summer of 1914 and continued uninterruptedly since that time.

Although there was already considerable evidence on the question of the effect of barberry on the rusts, it seemed desirable to do still further work. An attempt was therefore made to determine the possible role of barberry as a bridging host and also to determine its possible effect as a reinvigorator of the rust.

Material was obtained from as many different sources as possible for the work with cereals and grasses as intermediaries or bridging hosts. While much of the work with so many different strains might appear to be superfluous, nevertheless there has been some idea that strains of the same biologic form might differ somewhat parasitically. Naturally, therefore, it would be desirable to get data on as many different strains as possible.

Most of the work was done with the *tritici* and *secalis* forms because they are the most important economically in the spring-wheat States and because theoretically it seems probable that bridging should take place with these two forms on account of the close similarity of the rusts to each other in many respects. It has already been pointed out by the writers (*21*) that the *tritici* and *secalis* forms have many hosts in common. Thus, barley, various species of Elymus, Hystrix, Hordeum, and Agropyron are about equally congenial hosts for both rust forms. It would seem that if the idea of bridging is well founded, and if the host plants actually do exert a distinct and permanent effect on the rusts, these common hosts ought to unify rust strains which are grown on them.

Barley (*Hordeum* spp.) was used more than any of the other forms in attempted bridging on account of the fact that Freeman and Johnson

(9) found that it exerted such a pronounced effect on the rust, enabling any biologic form to increase its host range. The grasses were also used to a considerable extent, because both biologic forms of the rusts mentioned are so often found associated on them in the field. If rust changes rapidly, therefore, as a result of host influence, it would seem that the wheat stemrust and rye stemrust, growing on any one of these hosts for a number of urediniospore generations in the field, ought gradually to acquire the same parasitic capabilities.

The Agropyrons are especially interesting because the *tritici* form is able to attack some of them virulently (*A. tenerum* and *A. smithii*), while it can attack others, such as *Agropyron repens*, weakly or not at all. Here, then, there should be an opportunity to test the theory that taxonomic relationship determines the ability of the rust to pass from one host plant to another. If the stemrust of wheat (*Triticum* spp.) can not be transferred directly to *A. repens*, but can be developed normally on *A. tenerum* which is obviously more closely related to *A. repens* than to wheat, theoretically the rust should be able to pass from wheat to *A. repens* after it had first been transferred to *A. tenerum* or some other species of Agropyron closely related to *A. repens*.

Another problem is presented when a biologic form can attack a host plant weakly. If the theory of bridging hosts is a fact, certainly the rust must be easily changed by the host plants. Assuming, then, that individual plants of a given species vary in their susceptibility to the rust, it ought to be possible to increase the virulence of the rust on that particular host plant by successive inoculations with spores from the most vigorous uredinia. Or it ought even to be possible to increase the virulence of the rust by constant association with the uncongenial host. Rye is especially favorable for study in this respect. Individual rye plants vary very greatly in their susceptibility to *P. graminis tritici*. Some are entirely immune, others are almost immune, others are moderately susceptible, and still others are quite susceptible. Experiments were made to determine whether the rust from the susceptible plants when transferred to other rye plants could attack rye with greater virulence; also, whether it was possible to increase the virulence by simply transferring for a number of successive generations to rye. Similar conditions obtain when barley is inoculated with the *phleipratensis*, *avenae*, and *agrostis* strains, and experiments were made with other forms also.

As indicated in the historical summary, there seemed to be some evidence that changing the metabolism of the host might materially affect the parasitic capabilities of the rust. The writers therefore undertook further experiments along this line. The results of these experiments will be given in a separate paper not yet published.

If the biologic forms of *P. graminis* are easily changed, it seems reasonable to suppose, as previous experiments seem to have demonstrated

in a preliminary way, that this change brought about by a host plant should be manifested, not only in the parasitic capabilities of the rust, but also in the morphology. Extensive work was therefore done on this phase of the problem, and the results will be presented in a separate paper.

EXPERIMENTAL METHODS

The methods used in inoculating and incubating the plants were similar to those described by Stakman and Piemeisel (*21, p. 431–432*). On account of the fact that conclusions would be difficult to draw unless the experimental methods excluded to the greatest extent possible accidental infection, the utmost precautions were taken to prevent accidental infection. This is rather difficult when working under the conditions necessary in such an investigation. Unfortunately the pure-culture methods of bacteriology and mycology can not be successfully employed for rusts. However, it is possible to reduce the number of accidental infections to a very small minimum.

The seedling plants used in the experiments were grown under cages made of two layers of fine-mesh muslin separated by a dead-air space about an inch wide. Immediately after inoculation they were placed under bell jars, and as soon as the incubation period was over they were again placed under cages similar to those under which the seedling plants were grown.

Every precaution was taken to prevent infection from the outside by air-borne spores and to destroy all infected material as soon as possible. When accidental infection did take place, it could practically always be determined with certainty, although in a few cases it was not possible to do this. When, for instance, a leaf of wheat, as very rarely indeed happened, developed a normal rust uredinium as a result of inoculations with *P. graminis secalis* from rye, and when the spores in the uredinium morphologically and parasitically were in every way typical of *P. graminis tritici* spores, it seemed more reasonable to assume that accidental infection had taken place than to assume that bridging had occurred or that the rust had mutated. This conservatism in interpreting results seemed to be especially necessary when such a tremendous preponderance of evidence accumulated showing that bridging and mutations did not occur.

It was soon found also that the greatest precaution was necessary in being absolutely certain that the biologic form in use in a given experiment was absolutely pure before the results could be clearly interpreted. In making inoculations from barley and rye and many grasses from the field, both the *tritici* and *secalis* forms develop quite often. It is possible to inoculate all of the cereals and to obtain results which seem to show that only one biologic form is present, then to inoculate one of the common hosts for both forms, and then to find that a very small amount

of the other biologic form had been present on the original material, but was only given an opportunity to develop as a result of one or more transfers to the common host.

In experiments covering only a short period of time and with a small number of forms it might easily be concluded that bridging had occurred. The danger of drawing such conclusions, however, is clearly shown in diagrams 1 to 4, inclusive. Many cases of apparent bridging were seen, but in every case, with possibly one or two exceptions, it could be shown conclusively that this was due to the fact that more than one biologic form had been used.

The cereals used were the following, unless otherwise specified: Oats, Improved Ligowa (Minnesota 281); barley, Manchuria (Minnesota 105); wheat, Haynes Bluestem (Minnesota 169); rye, Swedish (Minnesota 2). Most of the grass seeds used were obtained by or through the Minnesota Seed Laboratory.

KEY TO TABLES I AND II AND DIAGRAMS 1 TO 10

The results of experiments to determine the effect of bridging hosts on the parasitism of the rust forms are given in Tables I and II and diagrams 1 to 10. The results of inoculations are usually given in the form of a fraction, the denominator indicating the total number of leaves inoculated and the numerator the number which developed uredinia. Whenever the presence of flecks is indicated, the number of leaves flecked is given after the semicolon following the fraction. Two types of diagrams are used—complete and condensed. Wheat, oats, barley, and rye are designated as W, O, B, and R, respectively. The names of the grasses are either written out in full or the key to the abbreviations is given in connection with each diagram. The sequence of transfers is indicated by dashes, proceeding from left to right. All of the plants indicated in the same vertical line after a dash were inoculated with the rust from the host immediately preceding the dash. Not all of the inoculations are indicated, since this would require altogether too much space. The essential ones, however, are indicated in the so-called complete diagrams, while summaries only are given in the condensed diagrams.

In the condensed diagrams the small number immediately following the symbol for cereal hosts or the names of the grass hosts indicates the number of urediniospore generations on that particular host. The fraction in parentheses indicates the result of inoculations which have been made during that period. The denominator gives the total number of plants inoculated and the numerator the number which became infected. The number of sets of inoculations is not indicated. Usually, although not always, the immune or highly resistant host was inoculated each urediniospore generation. For instance, "R_2—B_4—*Elymus canadensis*$_5$" indicates that two successive transfers had been made to rye followed by four successive transfers to barley and five to *Elymus canadensis*. These are spoken of as urediniospore generations. The transfers were usually made at intervals of approximately two weeks, so that each urediniospore generation represents about that length of time. "R_2 (wheat $\frac{0}{50}$)—B_4 (wheat $\frac{0}{25}$)—*Elymus canadensis*$_5$ (wheat $\frac{0}{35}$)" means that during the two generations on rye 50 leaves of wheat were inoculated, none of which became infected. One of the inoculations was probably made the first generation and the other the second generation. The rust was then transferred to barley and kept there for four

generations, during which 25 leaves of wheat were inoculated, none of which became infected. Usually this includes trials from most of the generations. The rust was then kept on *Elymus canadensis* for five generations, during which 35 leaves of wheat were inoculated without producing infection. "R_2—B_4 (wheat $\frac{0}{30}$)" would indicate that from the rust on barley 30 leaves of wheat had been inoculated, but none had been inoculated directly from rye.

When "strains" of rust are spoken of, the word is used in the sense of a biologic form with a certain history without any imputation that it is in any way different from a normal form.

The terms "intermediary host" and "bridging host" or "bridging species" are used in the theoretical sense—that is, hosts which apparently ought to cause bridging are spoken of as bridging hosts without any suggestion that they actually do cause bridging. The term "common host" is sometimes used. This means a plant which is a host for the biologic forms under discussion at that particular time.

EXPERIMENTS WITH BARBERRY AS A BRIDGING HOST

To test the ability of barberry to break down biologic forms and its capacity for "reinvigorating" the rust parasite, inoculation experiments were carried on during four æcial seasons. The teliospores were obtained from wheat, club wheat, oats and a few grasses. The common barberry (*Berberis vulgaris*) was used as the æcial host, and the æciospores produced were used for inoculating wheat, oats, barley, and rye. Four biologic forms of *P. graminis*—viz, *tritici*, *tritici-compacti*, *avenae*, and *secalis* were studied and the results obtained are given in Table I.

TABLE I.—*Results of inoculating cereals with æciospores of biologic forms of Puccinia graminis*

No.	Date.	Original sources of rust.	Biologic form.	Result.			
				W.	O.	B.	R.
1	May 25, 1914	Wheat	*tritici*	4/24	0/20	3/24	0/30
2	June 3, 1914	do	do	13/16	0/18	1/18	2/14
3	June 9, 1914	do	do	9/20	0/19	11/22	1/20
4	June 26, 1914	do	do	6/20	0/30	.....	1/13
5	May 8, 1915	do	do	5/10	0/14	10/14	0/12
6	May 23, 1915	do	do	15/34	0/35	2/28	1/14
7	June —, 1916	do	do	9/20	0/19	11/22	1/20
8	Apr. 11, 1917	Club wheat	*tritici-compacti.*	[a] 2/33,4	0/13	12/16	1/10
9	June 8, 1916	Oats	*avenae*	.....	12/14	[b] 1/29	.....

[a] *Puccinia graminis tritici-compacti;* very weak infection on wheat; normal infection ($\frac{5}{14}$) on club wheat.
[b] Minute uredinium.

TABLE I.—*Results of inoculating cereals with æciospores of biologic forms of Puccinia graminis*—Continued

No.	Date.	Original sources of rust.	Biologic form.	Result.			
				W.	O.	B.	R.
10	June 14, 1916	*Sporobolus cryptandrus.*	*secalis*	0/20	0/5	3/5	8/11
11	May 25, 1914	*Agropyron repens.*	.....do.....	0/17	0/20	4/19	3/20
12	June 3, 1914	.....do.....	.....do.....	0/18	0/19	5/16	2/15
13	June 9, 1914	.....do.....	.....do.....	1/22	0/21	0/15	8/22
14	.....	.....do.....	.....do.....	0/10	0/10	7/9	7/8
15	May 31, 1917	.....do.....	.....do.....	0/20	2/21	17/18	.....
16	July 2, 1917	*Agrostis stolonifera.*	.....(?)	9/41	4/27	.....	14/26

Barberry probably does not increase the host range of biologic forms commonly. If it did, æciospores collected in the field ought to infect cereals and grasses more indiscriminately than they do. On the other hand, the biologic specialization in the æcial stage is apparently the same as that in the uredinial stage. The percentage of infected leaves was often low, but this is often true when inoculations are made with æciospores. All of the results recorded in Table I, except No. 16, might equally well have been the result of inoculations with urediniospores, except that a larger percentage of inoculated leaves would have been infected.

The results shown in No. 16 can not be explained, unless accidental infection took place. The grass was collected in the fall and kept until the next July. The barberry had been kept in the greenhouse under a double muslin cage until inoculated and was again covered immediately after removal from the incubating chamber. Three biologic forms developed on the barberry; how they got there can not be stated with certainty.

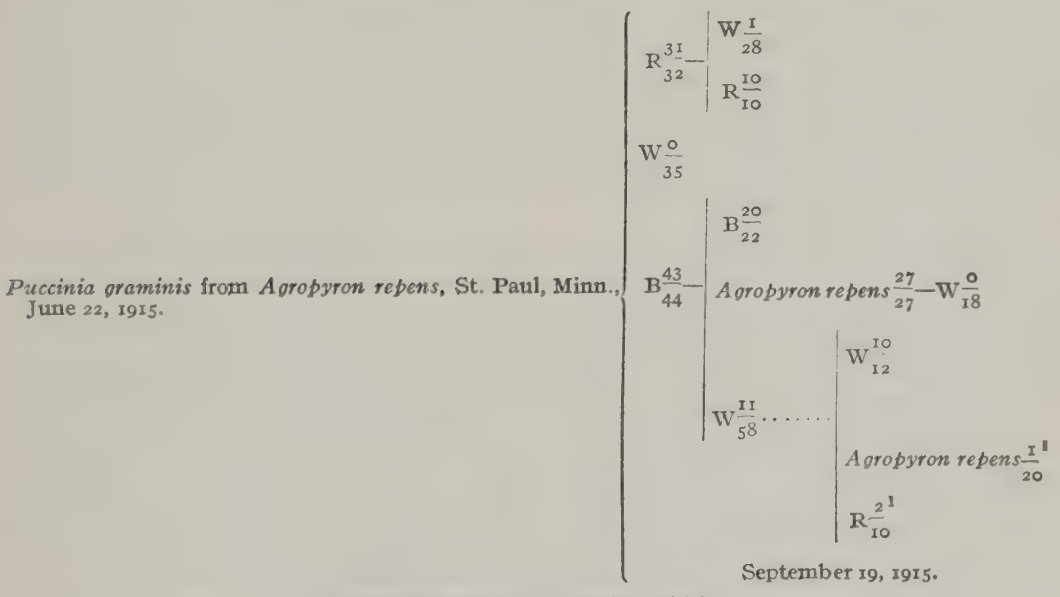
EXPERIMENTS WITH CEREALS AND GRASSES AS BRIDGING HOSTS

Many hosts equally susceptible to *P. graminis secalis* and *P. graminis tritici* were used as bridging hosts in attempts to change the parasitism of the two forms. Barley, various species of Elymus, Agropyron, Hordeum, and Bromus were used most. *Bromus tectorum* was used as

a possible bridging host for *P. graminis avenae*. Special care was taken to isolate the biologic forms whenever they were mixed, in order to avoid conflicting results and erroneous conclusions. The results of this phase of the work which extended for a period of over three years are given in diagrams 1 to 10 and in Table II.

It might have been concluded from the results shown in diagram 1 that barley acted as a bridge between *Agropyron repens* and wheat. It is shown quite clearly, however, by subsequent inoculations that it did not. The rust on the original quack-grass no doubt was mostly of the *secalis* form. It had probably been contaminated slightly in the field with some of the *tritici* form. Since there was only a small amount of the latter form, none developed on the wheat in the original inoculations. A very small amount developed on rye and more developed on barley. Therefore, when inoculations were made with the rust from barley, both the *tritici* and *secalis* forms were present, but they were separated in the third set of inoculations. A more complicated condition is shown in diagram 2.

DIAGRAM 1.—Results of inoculations with *Puccinia graminis* from *Agropyron repens* showing apparent bridging before biologic forms were isolated.



[1] Small uredinia; *P. graminis tritici*.

In diagram 2 both wheat and rye became infected when inoculated with the rust from *Hordeum jubatum*. The rust developed on wheat proved to be a pure strain of *tritici*. That developed on rye, however, consisted of both the *tritici* and *secalis* forms. Very clearly the original rust on *Hordeum jubatum* was a mixture of the *tritici* and *secalis* forms. The *tritici* form was isolated in pure form by transferring to wheat on which the *secalis* form did not develop. But the first generation of the rust on rye was still mixed, since *tritici* develops weakly on rye. Apparently the second generation of the rust on rye was pure *secalis*, since it did not develop on wheat, the leaves of which, however, died young. But, after having passed four generations on barley, the rust infected wheat normally and infected rye only weakly. This clearly looked like bridging. The more probable explanation, however, is that both the *secalis* and *tritici* forms again developed the second generation on rye. Since the wheat plants died young, the negative results recorded are not significant.

In the subsequent inoculations both *secalis* and *tritici* probably developed on barley, since a high percentage of inoculated leaves of *Agropyron repens* became infected. The *secalis* form, however, was eventually lost. This may have happened in two ways. The *tritici* form may have developed more rapidly than the *secalis;* or material from the leaves infected with *secalis* may not have been used in making inoculations It is quite probable that if rye had been inoculated earlier both forms would have been isolated.

From the R_1—W_1 material both biologic forms were isolated. This was puzzling at first, because wheat is not a host for the *secalis* form. The only plausible explanation seemed to be that spores of both biologic forms were placed on the wheat during inoculation and not all germinated in the moist chamber. A few viable *secalis* spores therefore remained on the wheat, and when these were transferred to rye, they germinated, causing infection. In order to ascertain whether this was possible, wheat was inoculated with the *secalis* form, kept in a moist chamber for 48 hours, and then kept on a greenhouse bench for about 10 days. No rust developed, but the inoculum was scraped from the leaves and used to inoculate rye plants. Infection resulted on a relatively large number of leaves, showing that the theoretical explanation advanced above was probably correct. These facts show that extreme caution is necessary in drawing conclusions when dealing with mixed forms of rust.

The most convincing proof that the rust forms, after isolation in pure form, could not be changed by host influences is furnished by the subsequent history given in diagram 2. The *tritici* form did not acquire new parasitic ability on account of its association with barley; nor did it increase in virulence on rye as a result of successive transfers. It will be noted that repeated unsuccessful attempts were made to develop a

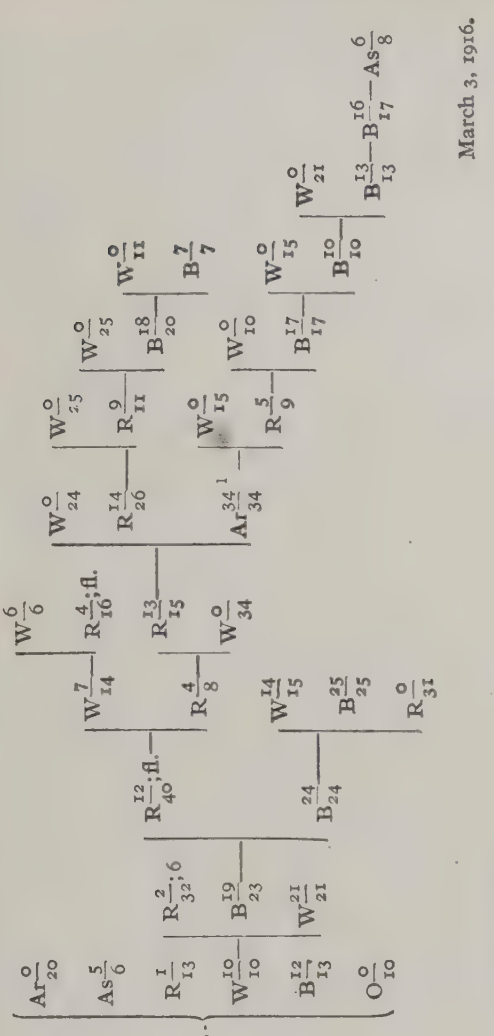
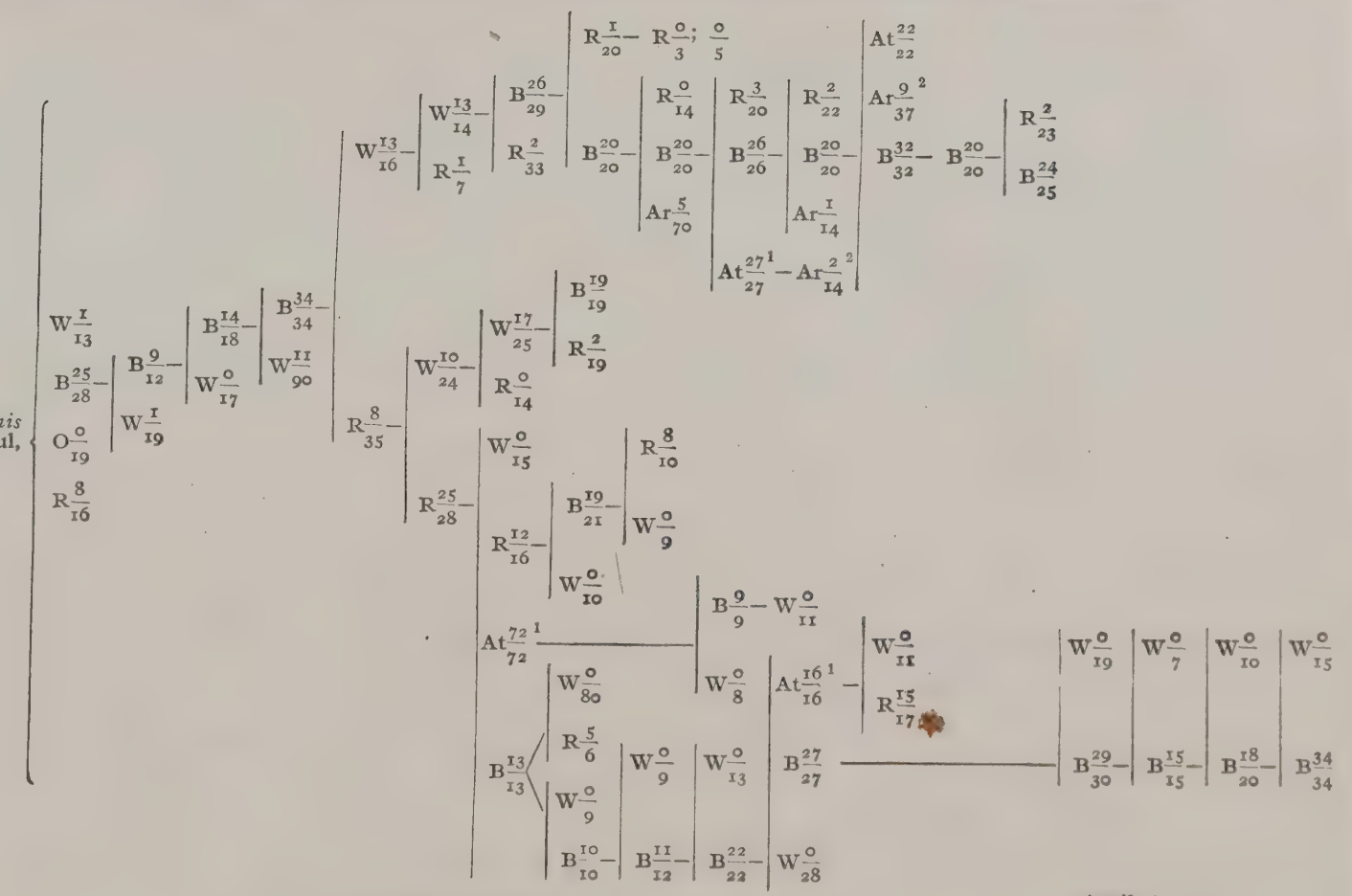

Puccinia graminis from Agropyron smithii, Mandan, N. Dak., August 27, 1915....
March 3, 1916.
Ar=Agropyro 1 repens.
As=Agropyron smithii.
[1] Small uredinia.

DIAGRAM 3.—Results of inoculations with *Puccinia graminis* from *Agropyron smithii*, Mandan, N. Dak.

strain capable of attacking rye normally. Diagram 2 shows also that the susceptible species of Agropyron used did not act as bridges to normally immune species of this genus. The rust was kept on barley for over a year, but it proved to be entirely stable and was therefore discarded. The *secalis* form likewise remained fixed after it was isolated and attacked its regular hosts vigorously, but could not be transferred to wheat or other normally immune hosts, a large number of which were inoculated, but the results of which are not given in the diagram for want of space.

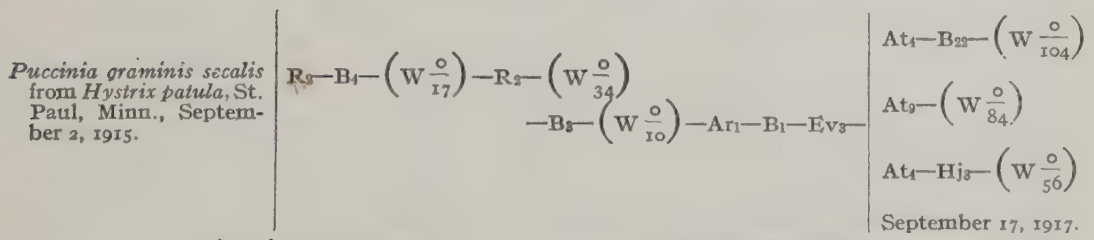
Erroneous conclusions might easily have been drawn from the results shown in diagram 3 if the inoculations had not been extensive. Here, again, the original rust was a mixture consisting mainly of the *tritici* form but including also a small amount of *secalis*. Viable spores of *secalis* were again carried through the moist chamber, and these infected barley, thus accounting for the later events. The *secalis* form was kept for about five months, but did not change.

DIAGRAM 4.—Effect of intermediary hosts on *Puccinia graminis secalis* and *P. graminis tritici* from *Agropyron cristatum*.


Puccinia graminis secalis and P. graminis tritici from Agropyron cristatum, St. Paul, Minn., September 10, 1915.
At=Agropyron tenerum.
Ar=A. repens.
[1] Heavy infection.
[2] Weak infection.
April 18, 1916.

The original rust was mixed here (diagram 4) also, and only after six successive sets of inoculations had been made were the *tritici* and *secalis* forms finally separated. Neither barley nor *Agropyron tenerum* acted as bridging hosts for the *tritici* form. The *secalis* form likewise remained fixed and did not acquire the ability to attack wheat after growing on either barley or *Agropyron tenerum.*

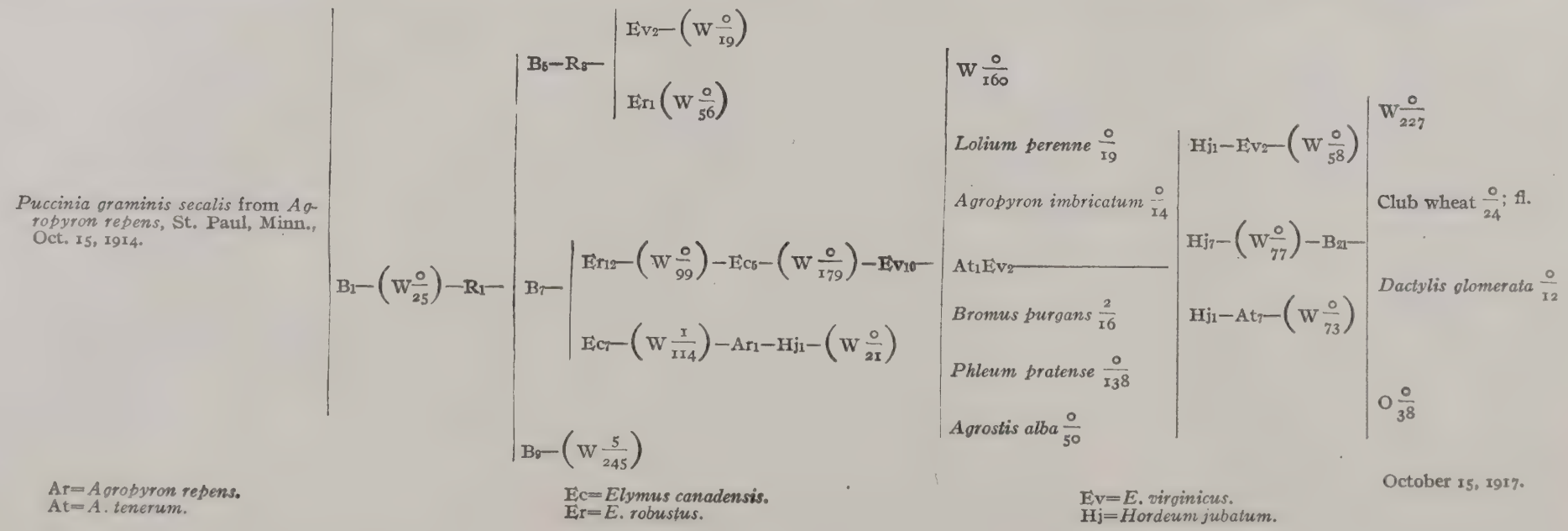
DIAGRAM 5 (condensed).—*Results of inoculations made with Puccinia graminis secalis* from *Hystrix patula.*



Ar=*Agropyron repens.*
At=*Agropyron tenerum.*
Hj=*Hordeum jubatum.*
Ev=*Elymus virginicus.*

The strain of *P. graminis secalis* from *Hystrix patula* was somewhat different from normal *secalis* strains (diagram 5). It was not as virulent on barley and rye, and the spores were somewhat smaller. Attempts were made to induce the rust to attack wheat by growing it on barley, *Elymus virginicus*, *Agropyron tenerum*, and *Hordeum jubatum.* None, however, acted as a bridge. The rust was kept for over two years, during which 18 sets of inoculations were made on wheat; but none of the 305 inoculated leaves became infected, except in one case, which was quite evidently an accidental infection with *P. graminis tritici.* However, the rust did not act normally and may have been a different biologic form. Some of the results were difficult to explain, and more work will probably be done with it.

DIAGRAM 6 (condensed).—Results of inoculations with *Puccinia graminis secalis* from *Agropyron repens* after various intermediary hosts.



Ar=*Agropyron repens.*
At=*A. tenerum.*

Ec=*Elymus canadensis.*
Er=*E. robustus.*

Ev=*E. virginicus.*
Hj=*Hordeum jubatum.*

This strain of *P. graminis secalis* was obtained (diagram 6) from *Agropyron repens* at St. Paul, Minn., on October 15, 1914, and was kept until October 15, 1917. It was confined to intermediary hosts for three years, during which time about 60 different sets of inoculations were made on wheat. About 1,800 leaves were inoculated, some of which are not recorded in diagram 6, and only six became infected. There is strong reason to suspect that these were accidentally infected with the *tritici* form, all occurring during the earlier period of work. Barley, *Elymus robustus*, *E. canadensis*, *E. virginicus*, *Agropyron tenerum*, and *Hordeum jubatum* were all used as intermediary or bridging hosts, but none of them enabled the rust to transfer to wheat, which remained practically immune throughout the entire period. Flecks very seldom developed. Since these hosts, which ought to act as bridging hosts, if any hosts act in this manner, did not enable the rust to transfer to wheat after three years of continuous culture, it seems quite improbable that such a change would take place quickly in nature.

In Table II a number of miscellaneous experiments are included. The most conclusive is No. 1, in which the *secalis* strain was kept continuously on barley for 16 successive urediniospore generations, covering a period of eight months. During this time 11 sets of inoculations were made on wheat, but none of the 264 leaves inoculated became infected, except one, which was very clearly shown to be accidental. In all of the other trials with the other strains of rust no infection whatever resulted on the wheat. It is quite clear, then, that under the conditions of these experiments neither barley nor *Elymus robustus* enabled the rust to bridge over to the normally immune wheat. This result could hardly be expected, because if such changes did take place in nature the biologic forms isolated from these hosts could not be as uniform as they are.

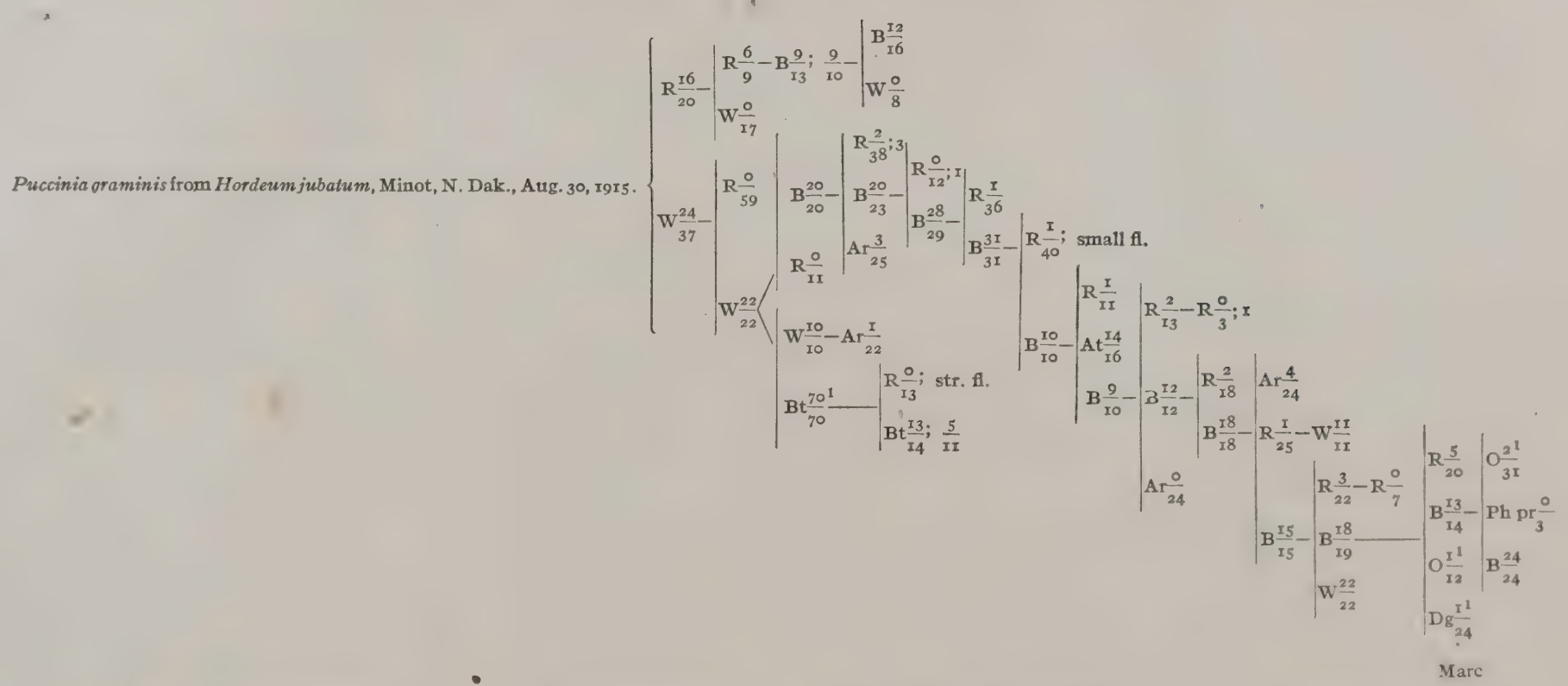
The last two experiments (No. 6 and 7), on the other hand, show the inability of barley to serve as a bridging host for the *tritici* strain, or to induce this rust to infect rye more vigorously than it ordinarily does.

TABLE II.—*Results of attempts to transfer Puccinia graminis secalis to wheat and P. graminis tritici to rye by using barley and Elymus robustus as intermediary hosts*

No.	Original host.	Place of collection.	Intermediary host.	Number of generations on intermediary host.	Time on intermediary host.	Number of trials.	Plant inoculated.	Number of leaves inoculated.	Number of leaves infected.
1	*Agropyron repens.*	Presque Isle, Me.	Barley.....	16	8 months..	11	Wheat.	264	[a] 0
2	Do.....	University Farm, Minn.	.....do.....	1	2 weeks...	1	...do....	59	0
3	Do.....	.....do.....	.....do.....	1	.....do.....	1	...do....	33	0
4	Do.....	Groveland, Minn.	.....do.....	1	.....do.....	1	...do....	30	0
5	Do.....	.....do.....	*Elymus robustus.*	1	.....do.....	1	...do....	11	0
6	*Hordeum jubatum.*	Berwick, N. Dak.	Barley.....	1	2½ weeks.	1	Rye....	42	0
7	Do.....	Lisbon, N. Dak.	.....do.....	4	1½ months	4	...do....	90	[b] 0

[a] One leaf accidentally infected with *P. graminis tritici*. [b] Four flecks in 2 out of 4 trials.

DIAGRAM 7.—Results of attempts to increase the infection capabilities of *Puccinia graminis* from *Hordeum jubatum*, Minot, N. Dak.



Ph pr= *Phleum pratense.*
At= *Agropyron tenerum.*

Bt= *Bromus tectorum.*
Ar= *Agropyron repens.*

Dg= *Dactylis glomerata.*

[1] Minute uredinia.

The rust from the *Hordeum jubatum* in this series (diagram 7) consisted originally of both the *tritici* and *secalis* forms. However, they were easily separated and remained fixed after separation. The *secalis* strain was kept only for a short time, since it proved to be an ordinary strain which failed to attack wheat either when transferred directly from rye or after having been transferred to barley. The *tritici* strain, however, was kept for some time in the expectation that it might be induced to attack rye more vigorously. Barley was used almost exclusively as a bridging host, but it is perfectly clear that it did not enable the rust to attack rye any more vigorously than the rust taken directly from wheat. Neither did it acquire the power to attack *Agropyron repens*, *Dactylis glomerata*, oats, or *Phleum pratense* any more readily than the rust taken directly from the wheat. It should be remembered that rye and *Agropyron repens* can often be attacked weakly by the *tritici* form, while oats and *Dactylis glomerata* are very rarely attacked, and timothy has so far proved entirely immune. One attempt was made to use *Bromus tectorum* as a bridging host, but it did not enable the rust to transfer to rye. In fact, no uredinia were developed, only a few strong flecks.

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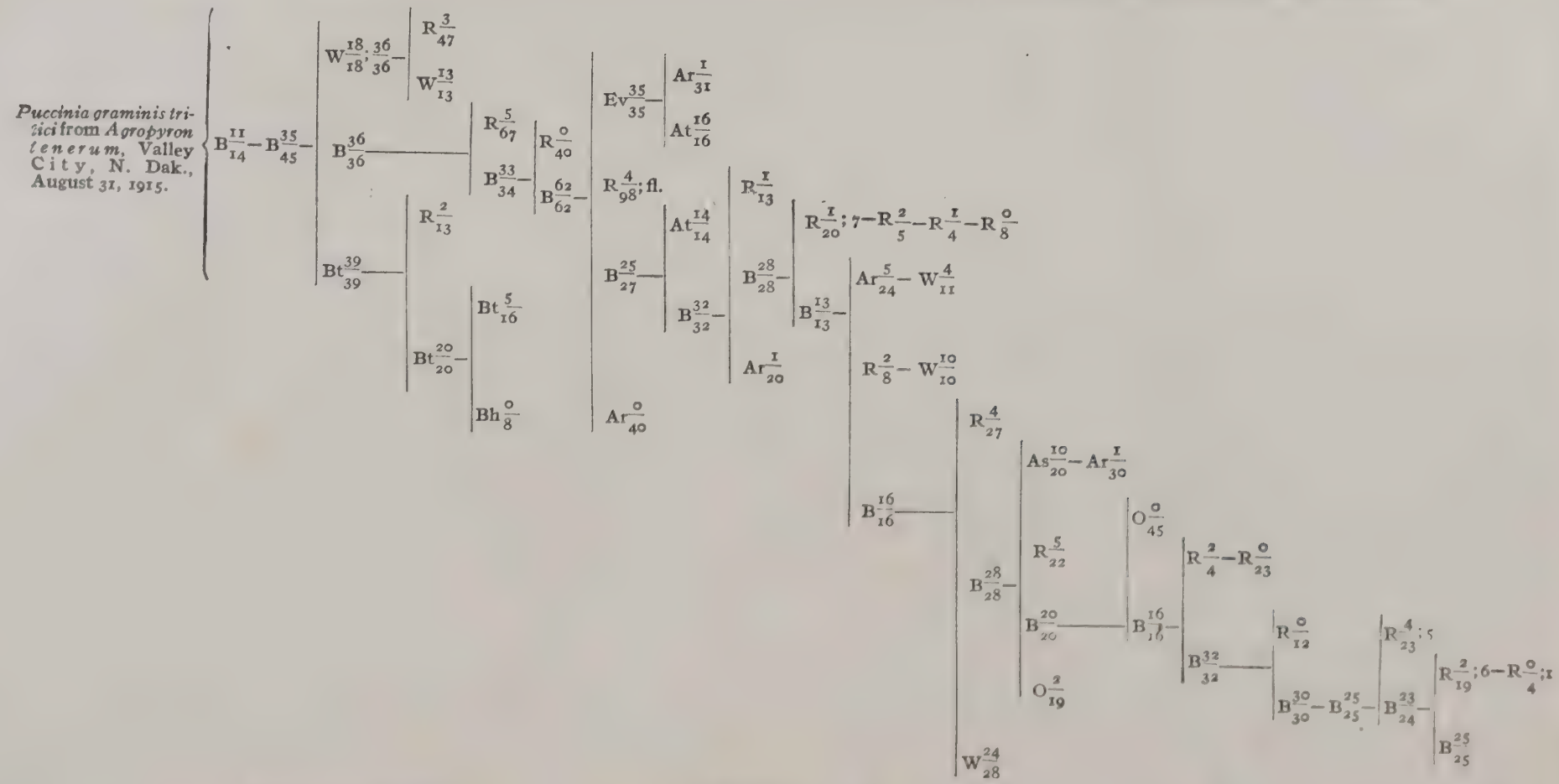
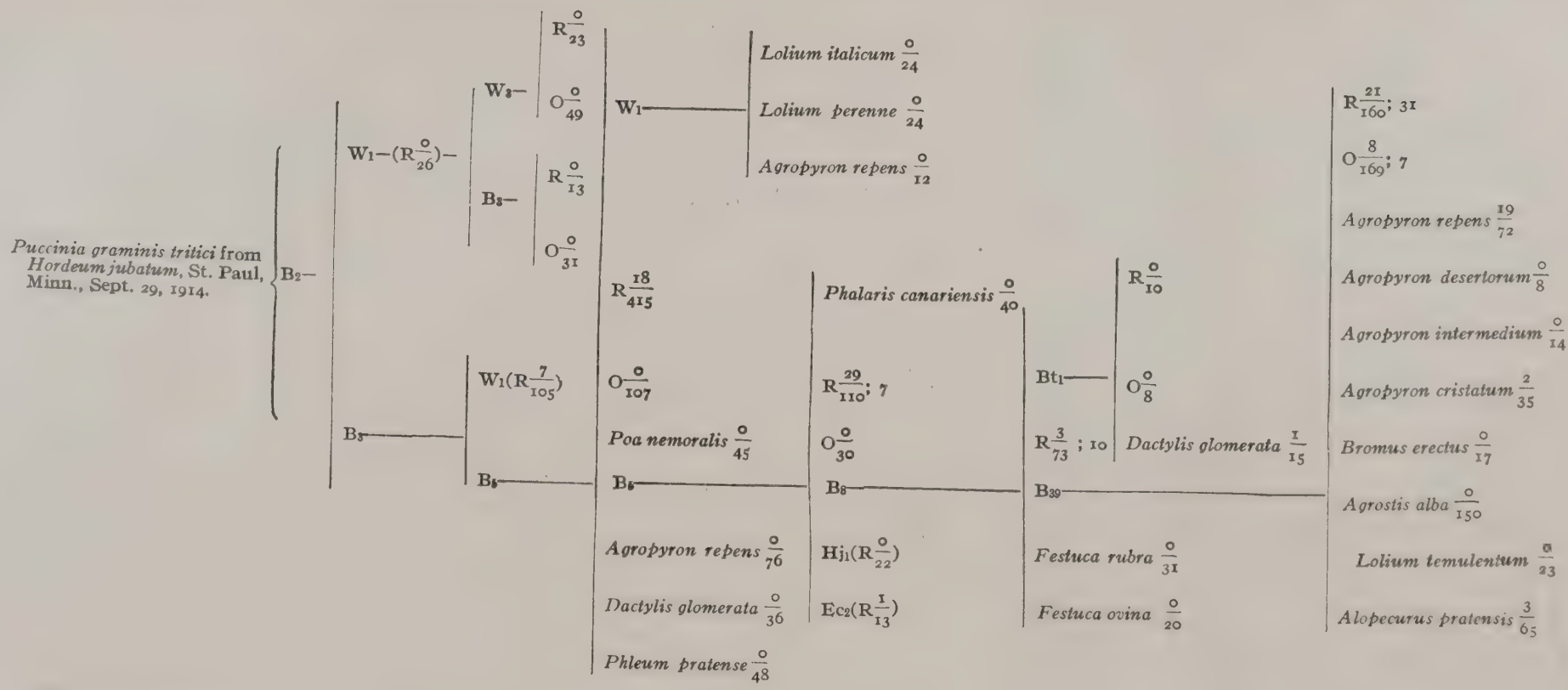
DIAGRAM 8.—Results of successive transfers of *Puccinia graminis tritici* from *Agropyron tenerum* to barley and other hosts.



May 23, 1916.

The strain of *tritici* used for the inoculations represented in diagram 8 was originally obtained from *Agropyron tenerum* at Valley City, N. Dak. It was one of the most vigorous *tritici* strains which has been obtained, but it was incapable of attacking rye, *Agropyron repens*, or oats with any degree of success, after having been kept on barley for a number of generations. The rust transferred readily to *Bromus tectorum* and was transferred from this host again to *Bromus tectorum* and *B. hordeaceus*, but it acquired no new power as a result of its sojourn on *B. tectorum*. *Elymus virginicus* was used in an attempt to get the rust to transfer to *Agropyron repens* but without success, only one small uredinium developing on one of the 31 inoculated leaves. *Agropyron smithii*, which is a congenial host, was inoculated and transfers then made to *Agropyron repens* in order to further test the taxonomic relationship theory. The results, however, were disappointing. Successive transfers were made to rye with urediniospores from a large uredinium which developed in one set of inoculations, but the rust died after three successive inoculations had been made.

DIAGRAM 9 (condensed).—Results of inoculations with *Puccinia graminis tritici* from *Hordeum jubatum* after various intermediary hosts.



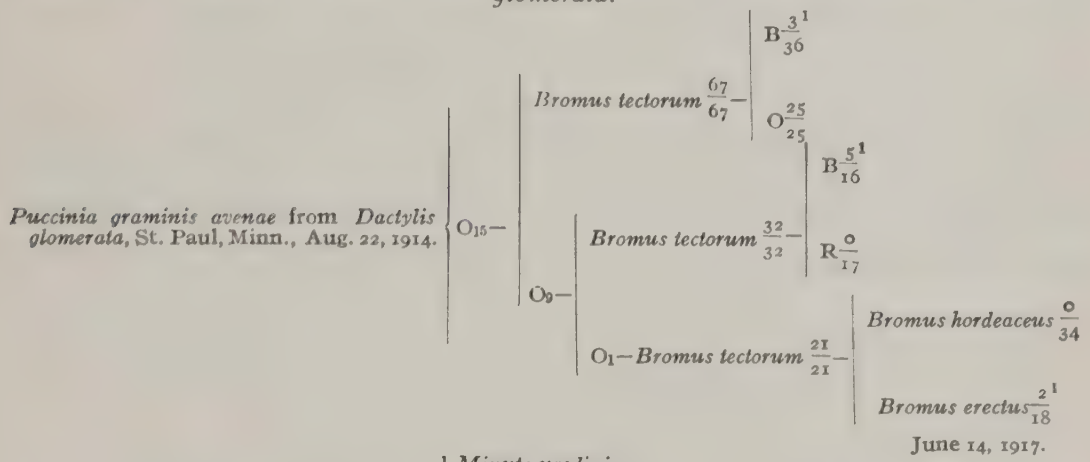
Bt=*Bromus tectorum.*

Ec=*Elymus canadensis.*

Hj=*Hordeum jubatum.*

The *tritici* strain used in this series of inoculations (diagram 9) was isolated originally from *Hordeum jubatum* at St. Paul, Minn., on September 29, 1914, and was kept in uredinial culture until June 15, 1917. Barley, *Elymus canadensis*, *Bromus tectorum*, and *Hordeum jubatum* were all used as intermediary hosts, but none of them enabled the rust to do anything which it could not do at the beginning of the experiment. The results of inoculations with the rust developed on *Bromus tectorum* are particularly interesting, since this grass is a host for the six common biologic forms of *P. graminis* in the United States. Theoretically when the *tritici* form was transferred to it, since it is a congenial host also for the *secalis* and *avenae* strains, it should have transferred to rye and oats. However, it did not.

DIAGRAM 10.—Results of inoculations with *Puccinia graminis avenae* from *Dactylis glomerata*.



[1] *Minute uredinia.*

The *avenae* strain represented in diagram 10 was isolated originally from *Dactylis glomerata* at St. Paul, Minn. It has been kept on oats for about three years. A large number of inoculations were made on various hosts during that time, but it has never performed differently from the rust on *Dactylis glomerata*. *Bromus tectorum* was used as a bridge in attempts to induce the rust to pass more readily to barley and rye, since it is a host for both the *tritici* and *secalis* forms as well as for the *avenae* form, but it is clear that it did not do this. The uredinia developed on barley were minute, and the percentage of infection was no greater than that which is obtained from transfers made directly from oats or any other host for the *avenae* form, a large number of which have been tried.

EXPERIMENTS ON THE ADAPTION OF BIOLOGIC FORMS TO SEMICONGENIAL HOSTS

In order to determine whether or not biologic forms are capable of adapting themselves to semicongenial hosts through constant association, many attempts were made to increase the virulence of the rusts on these hosts by means of continuous, successive transfers. The biologic forms of *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis* were studied and the results are given in Tables III to VI, inclusive.

TABLE III.—*Results of successive transfers of Puccinia graminis tritici from various sources to rye*

Original host.	Place of collection.	Previous history of rust.	Result.
Agropyron coninum..	Emerson, Manitoba.	W_1.....	$R\frac{3}{38}$; 10—$R\frac{4}{9}$; 2—$R\frac{1}{13}$; 2—$R\frac{0}{2}$
Agropyron cristatum.	St. Paul, Minn.....	B_4—W_2—B_3.....	$R\frac{3}{20}$—$R\frac{1}{7}$—$R\frac{2}{5}$—$R\frac{0}{2}$; $\frac{1}{4}$—$R\frac{0}{2}$
Agropyron tenerum..	Valley City, N. Dak.	B_3.....	$R\frac{1}{20}$; 7—$R\frac{2}{5}$—$R\frac{1}{4}$—$R\frac{0}{8}$
Do.....	.....do.....	B_{16}.....	$R\frac{4}{23}$; 5—$R\frac{2}{10}$; 5—$R\frac{1}{5}$; 2—$R\frac{0}{3}$
Do.....	Emerson, Manitoba.	W_1.....	$R\frac{4}{48}$; 13—$R\frac{4}{13}$; 7—$R\frac{0}{18}$; 11
Do.....	Glasgow, Mont.....	W_1.....	$R\frac{5}{25}$; 6—$R\frac{1}{27}$; 5—$R\frac{0}{2}$
Do.....	.....do.....	W_1.....	$R\frac{5}{44}$; 4—$R\frac{1}{7}$; 2—$R\frac{0}{2}$
Do.....	Crookston, Minn....	W_1.....	$R\frac{9}{30}$;11—$R\frac{4}{51}$; 19—$R\frac{1}{10}$;8—$R\frac{1}{9}$—$R\frac{0}{1}$
Elymus macounii....	Winnipeg, Manitoba	W_1.....	$R\frac{12}{46}$;37—$R\frac{9}{43}$;28—$R\frac{9}{20}$;11—$R\frac{4}{17}$;4—$R\frac{0}{3}$
Hordeum jubatum....	Two Harbors, Minn.	W_2.....	$R\frac{2}{20}$; 5—$R\frac{1}{11}$; 8—$R\frac{0}{3}$
Do.....	Grand Rapids, Minn.	W_1.....	$R\frac{10}{39}$; 6—$R\frac{8}{31}$; 6—$R\frac{3}{31}$; 10—$R\frac{0}{13}$; 3
Do.....	Minot, N. Dak.....	W_1.....	$R\frac{1}{11}$; 1—$R\frac{0}{2}$
Do.....	Cut Bank, Mont....	W_1.....	$R\frac{4}{34}$; 11—$R\frac{2}{30}$; 1—$R\frac{2}{5}$—$R\frac{1}{4}$;1—$R\frac{0}{5}$
Do.....	Williston, N. Dak...	W_1.....	$R\frac{2}{30}$; 4—$R\frac{0}{2}$
Do.....	Emerson, Manitoba.	W_1.....	$R\frac{6}{31}$; 14—$R\frac{2}{19}$; 11—$R\frac{0}{5}$
Do.....	Moorehead, Minn...	R_2—B_4—R_1—W_1.....	$R\frac{3}{14}$—$R\frac{3}{11}$—$R\frac{1}{3}$—$R\frac{0}{1}$
Do.....	.....do.....	R_2—B_4—R_1—W_2—B_1.	$R\frac{7}{26}$—$R\frac{7}{19}$—$R\frac{1}{6}$—$R\frac{1}{2}$—$R\frac{0}{2}$
Do.....	.....do.....	R_2—B_4—R_1—W_2—B_4.	$R\frac{12}{33}$—$R\frac{12}{20}$—$R\frac{3}{11}$;4—$R\frac{5}{24}$—$R\frac{1}{5}$;1—$R\frac{0}{5}$
Do.....	.....do.....	R_2—B_4—R_1—W_2—B_3.	$R\frac{1}{29}$; 9—$R\frac{2}{7}$; 2—$R\frac{3}{6}$; 3—$R\frac{0}{2}$
Hordeum vulgare.....	St. Paul, Minn.....	B_3.....	$R\frac{9}{21}$; 9—$R\frac{8}{22}$; 9—$R\frac{2}{10}$; 4—$R\frac{0}{3}$
Do.....	.....do.....	W_9.....	$R\frac{5}{28}$; 17—$R\frac{6}{25}$; 12—$R\frac{0}{2}$
Do.....	.....do.....	W_{10}.....	$R\frac{1}{13}$; 4—$R\frac{0}{4}$
Do.....	.....do.....	W_x[1].....	$R\frac{2}{7}$—$R\frac{0}{3}$

[1] x= Long-time association with host; number of urediniospore generations indefinite.

In Table III the results of a large number of attempts to increase the virulence of the *tritici* strain on rye by means of constant association with this host are given. It will be noticed that the rust was obtained from a number of different sources and from a number of different localities. The experiments covered a period of about three years. Whenever normal infection occurred on a rye plant the rust was transferred to other rye plants in the hope that it might prove to be a mutation or a tendency toward the differentiation of a strain of *tritici* capable of infecting rye normally. It will be seen, however, that this was not accomplished. The rust invariably gradually died out. Sometimes it seemed to increase in virulence as a result of successive transfers, often due to the abundance of infective material. At other times it apparently increased in virulence, owing merely to a change in experimental conditions. In several trials the rust had previously been on intermediary hosts for a number of generations, but this made no difference in the results. It so happened that some of the best results were obtained when the rust was taken directly from wheat. It was never possible to keep the rust on rye more than six generations. It simply became unthrifty and eventually failed to produce any uredinia, although excellent conditions for infection and subsequent development of rust were maintained.

TABLE IV.—*Results of attempts to increase the virulence of Puccinia graminis avenae by successive transfers to uncongenial hosts*

No.	Original host.	Place of collection.	Previous history of rust.	Result.
1	*Dactylis glomerata*....	St. Paul, Minn...	O_{29}.....	$B\frac{3}{24}-B\frac{0}{3}$
2	*Panicularia pauciflora*	Whitefish, Mont..	O_1.....	$B\frac{12}{14}-B\frac{0}{24}$
3	Do.....	.....do.....	O_1-Dg_1	Ph pr$\frac{7}{35}$−Ph pr$\frac{0}{8}$
4	*Dactylis glomerata*....	St. Paul, Minn....	O_6.....	$Bt\frac{10}{10}-Bt\frac{12}{14}-Bt\frac{5}{12}-Bt\frac{0}{4}$

Bt=*Bromus tectorum.* Dg=*Dactylis glomerata.* Ph pr=*Phleum pratense.*

The *avenae* strains used in the inoculations represented in Table IV were not able to develop increased virulence as a result of successive transfers on barley, *Phleum pratense*, or *Bromus tectorum*. The rust was kept on *B. tectorum* longer because this host is the most congenial of the three. However, it gradually died.

TABLE V.—*Results of successive transfers of Puccinia graminis phleipratensis to barley and oats*

No.	Original host.	Place of collection.	Previous history of rust.	Result.
1	*Festuca elatior.*	Sheridan, Wyo.	None....	$O\frac{7}{26}$; 4—$O\frac{1}{18}$—$O\frac{1}{2}$
2	Do.....	.....do.....	...do.....	$B\frac{9}{25}$; 16—$B\frac{0}{5}$; 5
3	Do.....	Bellingham, Wash.	..do.....	$O\frac{14}{31}$—$O\frac{16}{17}$—$O\frac{3}{13}$—$O\frac{1}{23}$—$O\frac{1}{9}$—$O\frac{0}{14}$
4	*Festuca pratensis.*	Pullman, Wash.	..do.....	$B\frac{14}{20}$—$B\frac{9}{14}$; 1—$B\frac{8}{8}$—$B\frac{0}{3}$; 1
5	*Phleum pratense.*	....do.....	...do.....	$B\frac{7}{13}$—$B\frac{7}{12}$; 5—$B\frac{1}{9}$; 4—$B\frac{1}{3}$—$B\frac{1}{6}$—$B\frac{1}{1}$—$B\frac{0}{1}$
6	Do.....	Ellensburg, Wash.	..do.....	$B\frac{18}{31}$—$B\frac{0}{20}$; 2
7	Do.....	Crawford, Nebr.	..do.....	$B\frac{10}{44}$—$B\frac{3}{23}$; 4—$B\frac{0}{4}$
8	*Dactylis glomerata.*	St. Paul, Minn.	Ph pr_6...	$O\frac{2}{13}$—$O\frac{2}{14}$—$O\frac{1}{8}$—$O\frac{0}{1}$
9	Do.....	.....do.....	None.....	$O\frac{14}{53}$—$O\frac{10}{26}$—$O\frac{9}{20}$—$O\frac{2}{16}$—$O\frac{0}{2}$
10	Do.....	.....do.....	...do.....	$O\frac{6}{12}$—$O\frac{3}{16}$—$O\frac{4}{7}$—$O\frac{8}{8}$—$O\frac{0}{5}$

Ph pr=*Phleum pratense.*

In Table V the results of attempts to build up *Puccinia graminis phleipratensis* from various sources on barley and oats are given. It is possible to maintain the rust for considerable periods of time, both on oats and barley, but the writers have never been able to keep it indefinitely. The number of uredinia became smaller, usually with each successive transfer, and the individual uredinia quite often decreased in size. Eventually so few spores are produced that only one or two leaves can be inoculated and these then fail to become infected.

TABLE VI.—*Results of successive transfers of Puccinia graminis agrostis to barley, oats, and rye*

No.	Original host.	Place of collection.	Result.
1	*Agrostis alba*.....	St. Paul, Minn.....	$B\frac{9}{16}—B\frac{8}{13}—B\frac{2}{12}—B\frac{3}{7}$; 2—$B\frac{0}{3}$
2	Do.....	.....do.....	$O\frac{6}{28}—O\frac{0}{11}$
3	*Agrostis stolonifera*.....	.....do.....	$O\frac{4}{22}—O\frac{2}{15}—O\frac{0}{7}$
4	Do.....	.....do.....	$R\frac{2}{25}—R\frac{0}{4}$

As seen from Table VI, *P. graminis agrostis* transfers with difficulty to barley, oats, and rye. The uredinia are always few in number and are practically always very small. It was impossible to increase the virulence of this rust by successive transfers to any of those hosts, although not a great number of experiments were made

GENERAL DISCUSSION

From the foregoing results it seems perfectly safe to conclude that if bridging and adaptation do occur, they occur rarely. Although all of the possibilities have not been exhausted it would seem that the experiments have been extensive enough practically to eliminate any idea of the possibility of sudden or even gradual changes in the rust under experimental conditions. Furthermore, as a result of extensive inoculations with biologic forms of *P. graminis* from a large number of hosts from widely separated localities (*21*), it seems that observational evidence corroborates the experimental evidence which the writers have obtained. The biologic forms obtained have remained pure and fixed after having once been isolated. It is true that when experiments are carried on for a short period of time only, there may appear to be distinct differences in the different strains of the same biologic form, but when the experiments are carried over a period of years it becomes quite evident that these differences are often due to experimental conditions. It seems quite probable that plus and minus fluctuations may occur, but that there is always a tendency to return to the normal. These plus and minus fluctuations may be induced by host influence or by environmental influence, but with an obligate parasite like *P. graminis* which can not be grown on a standardized medium but must be grown on living plants they are to be expected and the limits must be determined by extensive work.

Barley, which Freeman and Johnson (9) found to increase the range of parasitism of biologic forms has not been found to do this in the writers' experience. Attempts to induce bridging by means of this form have been made continuously for almost four years, and during all of that time no evidence whatever has been obtained that this host is able to change biologic forms. It is true that barley, as well as a number of the grasses, serves as a meeting point for a number of biologic forms and theoretically it seems as though it ought to change their parasitic capabilities. It also appears as though, if all of the common biologic forms of *P. graminis* could be kept on barley for a long period of time, they ought eventually to become practically uniform.

Although it is possible that rusts may change and new biologic forms may develop, it seems more probable that the change is either a very gradual one, extending over long periods of time, or that they change by mutation. No evidence of mutation, however, was obtained in the present investigation. The difference may be one of evolution as compared with experimentally induced change. For practical purposes, however, it seems perfectly safe to say that no certain and marked changes in biologic forms need be expected as a result of growing on bridging hosts; nor does it seem probable that biologic forms are able to gradually adapt themselves to semicongenial hosts by constant association with those hosts. The writers unsuccessfully tried to get evidence of such adaptation. Hybridization may possibly account for some unexplained phenomena and deserves investigation.

It still seems probable that rusts may change as a result of selecting strains from a given biologic form. While there is no positive evidence for this, it seems reasonable that biologic forms may be somewhat analogous to pure lines in genetics and that some forms may possibly be composite from which it is possible to isolate the component pure lines. This last supposition is theoretical only, and is prompted by a study of the *avenae*, *phleipratensis*, and *agrostis* forms. These are similar parasitically and the *avenae* form is variable morphologically, containing spores which after being isolated could be interpreted as being *agrostis* spores and others which might be determined as being *phleipratensis* spores. It is possible, although the experiments do not support the idea strongly, that a number of pure lines might be isolated from the *avenae* strain if extensive attempts were made.

Recently evidence has accumulated which seems to show that some of the apparent bridging obtained by previous investigators may have been due to the fact that several very closely related biologic forms may have been used in the experiments. The discovery of the differential hosts for these biologic forms is largely a matter of accident and the writers are of the opinion that all existing forms of *P. graminis* have not yet been discovered. The fact that several distinct biologic forms which attack various wheats are now known, is especially suggestive. A few varieties of wheat are differential hosts for these forms. Other closely related forms may exist and unless the investigator is lucky in stumbling

onto them, all sorts of puzzling results may be obtained and erroneous conclusions may be drawn.

Biologic forms must have originated in some way and the forces which induced their origin may still be operative but it seems probable that these forces operate so slowly that they do not affect the practical problems of controlling rusts of economic importance. It is highly important, however, that the geographic distribution of biologic forms be ascertained and their relation to the varieties grown or bred for those regions be intensively studied. When this has been done many of the apparently strange and inexplicable phenomena of rapid change in rust resistance will undoubtedly be explained.

SUMMARY

(1) Barberry does not increase the host range of biologic forms; nor does it act as a reinvigorator of the rust. The biologic specialization in the aecial stage is apparently the same as that in the uredinial stage.

(2) Differential hosts must be used to isolate biologic forms from mixtures before conclusive experiments can be made with bridging hosts.

(3) In experiments with a small number of biologic forms and extending over a short period of time there is danger of erroneously concluding that bridging has occurred.

(4) Many hosts equally susceptible to *P. graminis secalis* and *P. graminis tritici* were used as bridging hosts in attempts to change the parasitism of the two forms. Barley, various species of Elymus, Agropyron, Hordeum, and Bromus were used most.

(5) *Puccinia graminis secalis*, which does not attack wheat, but does infect barley readily was cultured on barley and other theoretical bridging hosts continuously for three years during which time more than 2,000 wheat plants were inoculated. The rust acquired no new parasitic capability on account of its association with barley.

(6) *Puccinia graminis tritici* attacks wheat readily, but can attack rye only weakly. Barley is easily attacked. The rust was confined to barley for about 32 months but it never acquired the power of attacking rye more readily than rust taken directly from wheat.

(7) Several species of Elymus, Agropyron, Hordeum, and Bromus were used as bridging hosts for both the *secalis* and *tritici* forms; but no bridging resulted.

(8) Attempts to change the parasitism of *P. graminis avenae* by means of bridging hosts were also unsuccessful.

(9) The taxonomic relationship theory of bridging was tried. If plant C can not be attacked by the rust from a taxonomically distant host plant A, it can not be attacked after the rust has been grown on a form B intermediate taxonomically between A and C.

(10) No one so-called bridging host nor any combination of such hosts enabled any biologic form tried to infect naturally immune plants nor to infect a highly resistant plant more readily.

(11) Many attempts were made to increase the virulence of biologic forms on resistant hosts by successive transfers to these hosts. *P. graminis triciti*, *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis* were used. The results indicated that rust forms do not gradually adapt themselves to resistant or semicongenial hosts.

(12) Biologic forms seem to be roughly analogous to pure lines. Plus and minus flucutations may occur, but there is always a tendency to return to normal.

(13) It is possible but not demonstrated that some biologic forms may be mixtures from which "pure lines" can be isolated. *P. graminis avenae* is a possibility.

(14) The facts given in this paper do not support the conclusions of previous workers that the pathogenicity of biologic forms is easily changed by host influence.

(15) From the practical standpoint the constancy of biologic forms is of great importance. Breeding for rust resistance can proceed with considerable assurance that the same rust will not adapt itself quickly to new varieties.

(16) Biologic forms may have arisen either by mutations or by gradual process of evolution. These processes may be operative yet, but the writers have not been able to detect any mutation nor to induce perceptible evolutionary changes experimentally. The possible rôle of hybridization will be investigated.

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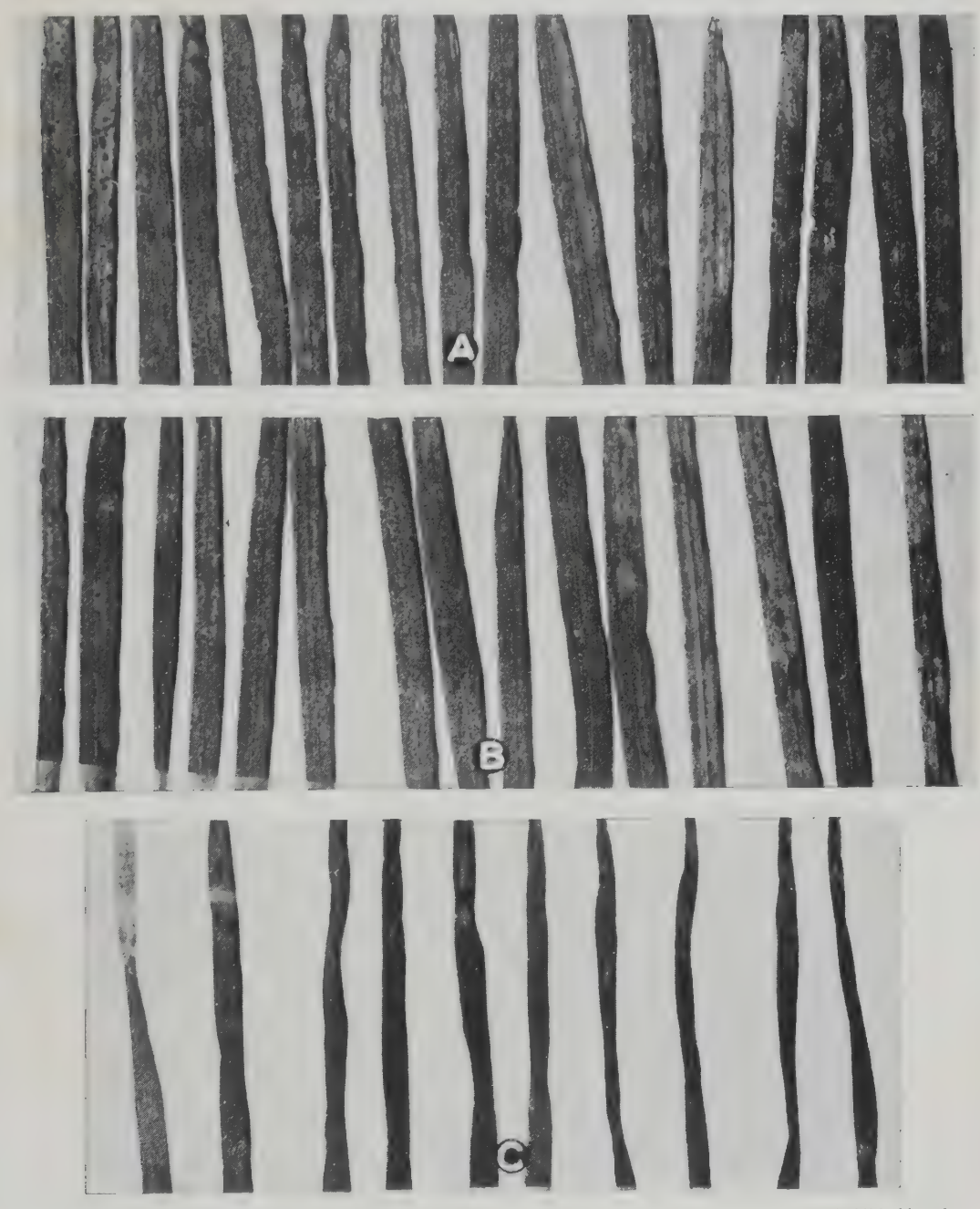
PLATE 17

A, B.—*Puccinia graminis tritici* from *Hordeum jubatum* (Moorhead, Minn.) on rye after having previously spent 2 urediniospore generations on rye, 4 on barley, 1 on rye, 2 on wheat, and 5 more on barley. Twenty-one out of thirty-two blades inoculated became infected; the uredinia produced were very small and surrounded by hypersensitive areas.

C.—*Puccinia graminis tritici* from *Hordeum jubatum*, originally from Moorhead, Minn., but with subsequent history of R_2—B_4—R_1—W_2—B_5—R_1. (See fig. A.): Normal infection on wheat.


A
B
C

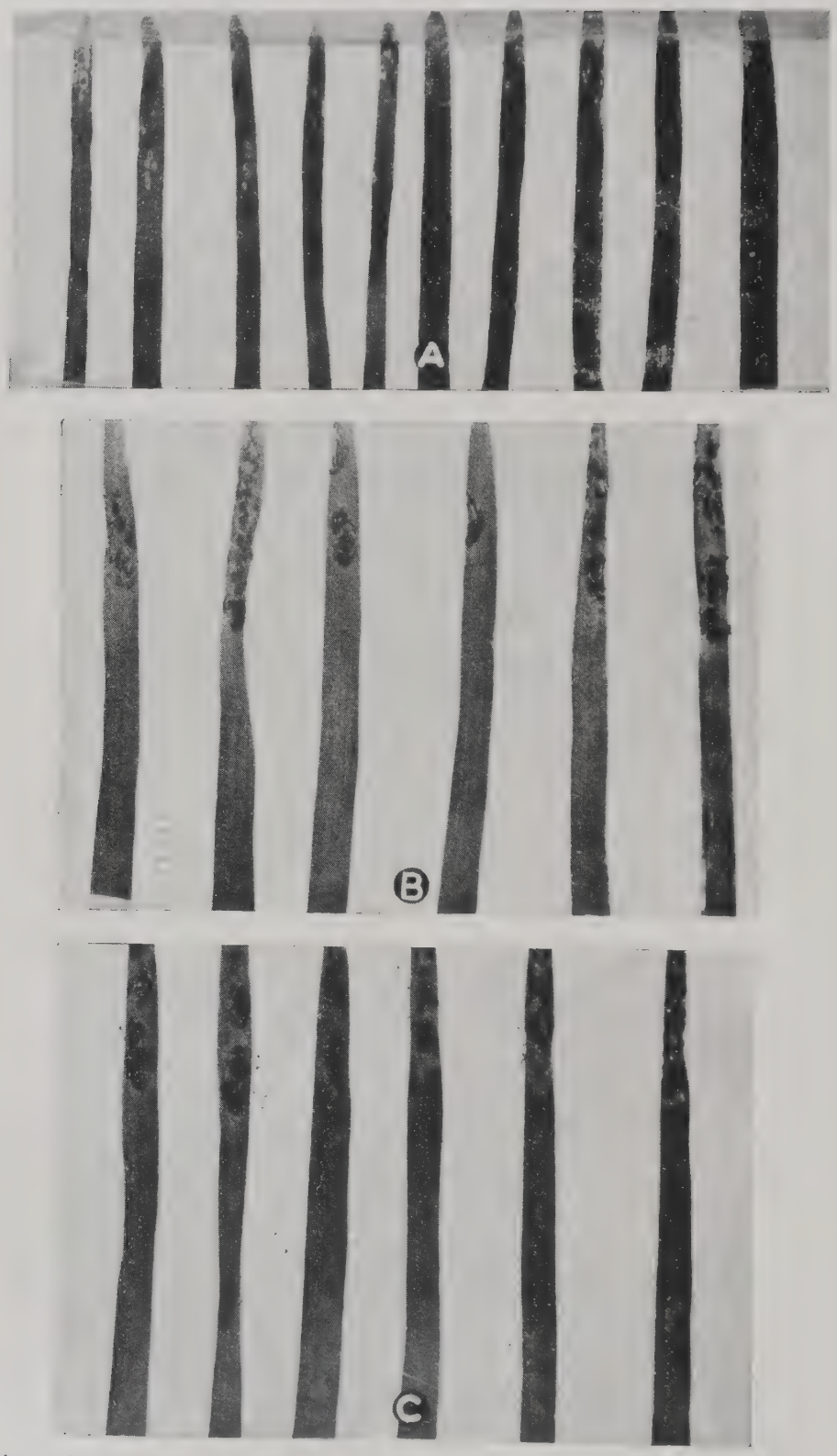

A
B
C

PLATE 18

A.—*Puccinia graminis tritici* from *Hordeum jubatum*, originally from Moorhead, Minn., but with subsequent history of R_2—B_4—R_1—W_2—B_5—R_1: Small uredinia and sharp flecks on rye.

B, C.—*Puccinia graminis tritici* on wheat. Normal development of uredinia produced by inoculating with urediniospores of the following life history: B. *P. graminis tritici* (St. Paul, Minn.) B_{31}—*Agropyron tenerum*$_1$—*A. repens*,—W_1—*A. repens*$_1$. C. *P. graminis tritici*(St. Paul, Minn.) B_{31}—*Agropyron tenerum*$_1$—*A. repens*$_1$—W_1—*A. tenerum*$_1$.

EXPERIMENTS IN FIELD TECHNIC IN PLOT TESTS [1]

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INTRODUCTION

In summarizing the replies to a circular letter sent out to Experiment Stations in the United States and in Canada, one is impressed with the wide variations in the size and shape of the plots and in the width of alley between plots in variety, cultural, rotation, and fertility work. The general size of plots varies from $\frac{1}{120}$ to $\frac{1}{8}$ acre and in shape from 3 to 33 feet wide and 36 to 272.25 feet long. The width of alley between plots varies from none to 8 feet. Twenty-two STATIONS report the removal of end borders from plots, and nine report no alleys, with grain removed to form pathway or the removal of one side border drill row from either side of each plot.

This lack of uniformity in experimental technic in plot tests, particularly with regard to alley effect, which involves shape and size of plot, raises several questions, among which are the following:

(1) How far within plots is alley effect operative?

(2) What is the increase in yield due to alley effect?

(3) In plots surrounded by alleys, is the effect of the additional space the same on all varieties?

Data obtained at University Farm in 1917 from which to determine the most desirable methods in plot variety testing and cultural trials form the basis of this paper. Although the results are for one season only, they appear to be sufficiently conclusive to warrant their publication. It is hoped that similar work may be done elsewhere, and the collected data serve as a basis for the adoption of more uniform methods in plot tests.

REVIEW OF LITERATURE

Montgomery (*6*)[3] suggests two methods of obviating competition between the larger and more rapidly growing varieties of wheat and oats grown in 8- and 10-inch rows—namely, to plant only similar varieties in adjacent rows and the use of block plots. The possible effect on the

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[2] The authors wish to acknowledge their appreciation of the assistance given by Mr. R. J. Garber, Assistant in Farm Crops, in compiling the data.

[3] Reference is made by number to "Literature cited," p. 270.

yields of different varieties when grown in plots surrounded by alleys is not considered. Shape seemed to be of little importance in plots not surrounded by alleys.

Barber (*1*) noted that plants in the borders of plots surrounded by alleys were more thrifty as indicated by a greater number of culms per plant, a longer period of growth and a higher yield of grain. Yields obtained at the Maine Agricultural Experiment Station for individual oat plants utilizing 36 square inches of space as compared with others occupying 6 square inches showed that the former yielded four times as much grain as the latter. Additional evidence of the increased yield of plants with greater space for development is quoted from Wacker (*10*).

Based on the investigations of Ten Eyck (*9*) and Rotmistrov (*8*), Barber (*1*) makes the estimate that plants growing in an area 6 inches wide within the borders of plots receive benefit from the adjacent alleys. Tables are given showing that the nearer a plot is to the form of a square, the lower is the percentage a 6-inch strip around the border of a plot to its total area. From this it is concluded that (*1*) shape as well as size of plot must be considered in variety tests, and (*2*) that square plots give more accurate results in variety testing than rectangular plots of the same size.

Mercer and Hall (*5*) conclude that, based on data secured from plots removed from large fields and therefore not surrounded by alleys, there is practically no difference in the variability of yields obtained from oblong and square plots of the same size.

Jardine (*2*, *3*) mentions that the practice of removing the outer drill rows of field plots to eliminate alley effect is followed by the Kansas Experiment Station.

Love and Craig (*4*) show that the same varieties of wheat and oats average 36.51 per cent higher in yield when grown in plots $\frac{1}{200}$ acre in size as compared with the yields in rod rows from which the end borders have been removed before harvesting. The shape of the plots is not given. Differences in stands under the two systems are mentioned.

MATERIAL AND METHODS OF EXPERIMENTATION

In the final variety tests of oats, wheat, and barley conducted on University Farm in 1917, each variety was replicated three times, thus making four plots of each. The plots were 8.5 by 132 feet with 16-foot roadways seeded to grass between each two series and 18-inch alleys between each two plots. The 18-inch alleys represented areas not included within the margins of the adjacent plots. Of each variety, then, there were available for the determination of alley effect four plots made up of seventeen 6-inch drill rows and 1/38.82 (approximately $\frac{1}{40}$) of an acre in size. Eleven varieties of oats, five varieties of wheat, and four varieties of barley were included in the tests.

In the spring the plots were sown the full width of the series, which is usually 134 feet. In order to facilitate the removal of end borders accurately at harvest time, shortly after seeding in the spring, two galvanized-iron wires were stretched exactly 132 feet apart across the ends of the plots. The distance between the wires was checked every fifth plot along the series and the wires anchored securely. A few days before harvest time the grain on the ends of all the plots outside of the lines marked by the two wires was cut and discarded.

At harvest time the drill row next to the alley on either side of each plot was removed by hand, bound and tagged separately. These are referred to as outside border rows. The second drill rows on either side of each plot were next removed by hand and bound and tagged separately. These are referred to as inside border rows. The 13 remaining 6-inch drill rows were then harvested with the self-binder. In harvesting the central 13 rows with the binder there probably was somewhat more shattering of the grain than occurred in harvesting the border rows by hand.

The sizes of the different areas from which yields were determined are summarized for convenience.

Number of 6-inch drill rows.	Dimensions of areas.	Part of an acre.
1	6 inches×132 feet	1/660
13	6.5 feet×132 feet	1/50. 77
15	7.5 feet×132 feet	1/44
17	8.5 feet×132 feet	1/38. 82

The grain from the portions of the plots which were harvested separately were threshed with a small machine and the yields of each computed. The number of pounds of grain threshed from the central 13 rows and from the two inside border rows of each plot were then added, and from this the yields of the plots with one border row removed from either side of each plot were computed. The number of pounds threshed from all parts of each plot were then totaled, and the yields for the plots with no border rows computed.

DISTANCE WITHIN PLOTS AT WHICH PLANTS ARE SUBJECT TO BORDER EFFECT

For the purpose of determining the distance within plots affected by adjacent alleys, the yields of the outside border rows, the inside border rows, and the average of the central 13 rows of four plots of each variety of oats, wheat, and barley are available. These yields are summarized in Table I.

TABLE I.—*Average yield, in bushels per acre, of oats, wheat, and barley harvested from border drill rows spaced 6 inches apart removed from either side of plots 8.5 by 132 feet and from the central 13 rows remaining after the removal of the border rows*

Source.	Oats.		Wheat.		Barley.	
	Number of rows or plots.	Yield per acre.	Number of rows or plots.	Yield per acre.	Number of rows or plots.	Yield per acre.
		Bushels.		*Bushels.*		*Bushels.*
Outside border rows.....	88	131.97	40	55.00	32	97.73
Inside border rows.....	88	87.95	40	40.98	32	64.46
Central 13 rows.....	44	71.37	20	27.45	16	42.87

The actual yield of the outside border rows is for oats 83.5 per cent, for wheat 100.4 per cent, and for barley 123.3 per cent greater than the average for the central 13 rows for the same varieties. For the three crops represented, the outside border rows yielded 102.1 per cent higher than the average for the central 13 rows of the same varieties.

For oats, the inside border rows averaged 23.23 per cent, for barley 50.36 per cent, and for wheat 49.29 per cent higher in yield than the average for the central 13 rows. For the three crops there was an average increase of 41 per cent in the yield of the inside border rows as compared with the average of the central 13 rows of the same plots. The outside and inside border rows on either side of each plot, together averaged 143.1 per cent higher in yield than the average of the central 13 rows in the same plots. Thus, plants growing in the two outside and two inside border rows on either side of each 18-inch alley, kept reasonably free from weeds, appear to have utilized this area in addition to the space allotted to them within the plots nearly as well as though it had been regularly occupied by three drill rows of plants. This suggests that, within certain limits, width of drill row is a negligible factor in seeding as long as other conditions are uniform. The possible effect of the alleys on the plants farther than 12 inches within the margins of the plots was not determined, but further work is in progress with this object in view.

The plants in the outside border rows particularly were still somewhat green when those in the interior rows were mature. The oat and barley varieties were harvested as soon as the plants in the interior of the plots were mature. By the time the wheat plots could be harvested the plants in the border rows appeared as mature as those in the central rows. The weights per bushel of the oats and barley from the central 13 rows averaged 31.60 and 40.60 pounds, respectively, as compared with 30.08 and 39.50 pounds for that from the outside border rows. There was practically no difference in the average weights per bushel of the oats and barley from the inside border rows as compared with that from the central 13 rows. No differences in weight per bushel were found in the wheat harvested from the three areas of the plots.

The results for oats, wheat, and barley indicate that in plots surrounded by alleys the effect of the additional space extends to the plants occupying areas 12 inches within the margins of the plots and possibly farther. The higher yield of the plants in the outside border rows appears to be due to better nutrition, as is indicated by later maturity.

EXTENT OF INCREASE IN THE YIELD OF PLOTS DUE TO ALLEY EFFECT

From the foregoing it is obvious that the extent of the increase in the yields of plots surrounded by alleys depends upon the relative proportion of the area of the border strip occupied by plants subject to the effect of the additional space to the total area of the plot. In Table II are summarized the percentages (1) of the total areas of plots of different sizes but of the same shape and (2) of plots of the same size, but varying in shape contained in borders 12 inches wide (a) on two sides of plots only and (b) on the ends and sides of plots.

TABLE II.—*Relation of a 1-foot border within a plot to its total area*

Approximate size of plot (fraction of acre).	Dimensions of plot in feet.	Approximate shape of plot.	Area of plot.	Area [a] of border, 1 foot wide on sides of plots only.	Percentage of total area of plot in border, 1 foot wide on sides of plot only.	Area of borders 1 foot wide on ends and sides of plots.	Percentage of total area of plot in border, 1 foot wide on ends and sides of plot.
	Feet.		*Sq. ft.*	*Sq. ft.*		*Sq. ft.*	
1/440	3×33	1:11	99.0	64	64.65	68	68.69
1/220	3×66	1:22	198.0	130	65.66	134	67.68
1/220	6×33	1:5.5	198.0	64	32.32	74	37.37
1/110	3×132	1:44	396.0	262	66.16	266	67.17
1/110	6×66	1:11	396.0	130	32.83	140	35.35
1/110	12×33	1:2.75	396.0	64	16.16	86	21.72
1/55	24×33	1:1.375	792.0	64	8.08	110	13.89
1/160	8.5×33	1:4	280.5	64	22.82	79	28.16
1/80	8.5×66	1:8	561.0	130	23.17	145	25.85
1/80	17.0×33	1:2	561.0	64	11.41	96	17.11
1/40	8.5×132	1:16	1,122.0	262	18.89	277	24.69
1/40	17×66	1:4	1,122.0	130	11.59	162	14.44
1/40	34×33	1:1	1,122.0	64	5.70	130	11.59
1/20	17×132	1:8	2,244.0	262	11.68	295	13.15
1/20	34×66	1:2	2,244.0	130	5.79	196	8.73
1/10	34×132	1:4	4,488.0	262	5.84	328	7.31
1/10	68×66	1:1	4,488.0	130	2.90	264	5.88

[a] In computing the area of a strip 12 inches on the sides of a plot only, 2 square feet, which belong to the end borders, must be deducted from the total area in the two sides strips.

An examination of the percentages given in Table II shows that the relation of the areas affected by the additional space afforded by alleys to the total area of plots is dependent upon both the shape and the size of the plots.

Plots $\frac{1}{440}$ and $\frac{1}{110}$ acre in size and each having a width to length ratios of 1 to 11 have in ends and sides 68.69 and 35.35 per cent, respectively, and in the sides only 64.65 and 32.83 per cent, respectively, of their total area in a 12-inch strip within their margins. Likewise, plots $\frac{1}{40}$ acre in size with a ratio of 1 to 4 for width to length have only approximately half as great areas in 12-inch strips within their margins as those $\frac{1}{160}$ acre in size and of the same shape.

Increased size then in plots of the same shape reduces the area exposed to alley effect. Increase in size of plots where shapes are widely different may not bring about a reduction of the area in the marginal strip exposed to alley effect. As an example of this, plots $\frac{1}{55}$ acre in size and 24 by 33 feet compared with those $\frac{1}{40}$ acre in size and 17 by 66 feet have, respectively, 13.89 and 14.44 per cent of their total area in a 12-inch marginal strip.

As shown in Table II, plots $\frac{1}{220}$ acre in size but having width to length ratios of 1 to 22 and 1 to 5.5 have 67.68 and 37.37 per cent, respectively, of their total area in 12-inch marginal strips. Plots $\frac{1}{40}$ acre in size with the width to length ratios of 1 to 16 and 1 to 1 (approximate) have 24.69 and 11.59 per cent, respectively, of their areas in 12-inch marginal strips. The nearer plots approach the form of a square, the lower the percentage of their total area is exposed to border effect (*1*).

A consideration of the percentages given in Table II brings out the fact that yields from the same varieties or treatments obtained under like conditions, but from different-sized plots, surrounded by alleys are not comparable.

If end and side border effect are similar, it should also be noted in this connection that the removal of end borders only from plots of several different sizes surrounded by alleys may render yields from them still less comparable. A single example using the data included in Table II for plots $\frac{1}{40}$ acre in size, but the width to length approximately 1 to 16 and .1 to 1, respectively, will suffice to make this point clear. With no borders removed the percentage of a 12-inch strip within the margins of the plots to the total area of each are 24.69 and 11.59, and with end borders removed, 18.89 and 5.70, respectively. With no borders removed the percentage of the plots 8.5 by 132 feet exposed to alley effect is approximately twice as great as for those 34 by 32 feet. When the end borders are removed, these percentages are 18.89 and 5.70, respectively, the one being approximately three times as great as the other.

In Table III are given the yields of oats, wheat, and barley grown in plots of the same length, but varying in width. The yields for each crop from the $\frac{1}{110}$-acre plots are considerably higher than those from the $\frac{1}{40}$-acre plots and the yields from the $\frac{1}{40}$-acre plots are somewhat higher than those from the $\frac{1}{10}$-acre plots.

TABLE III.—*Average yields of Ligowa oats (Minnesota 281); Haynes Bluestem wheat (Minnesota 169), and Manchuria barley (Minnesota 105), grown in plots of the same length, but varying in width and border rows removed*

Number of plots.	Approximate size of plots (fraction of acre).	Dimensions of plots.	Crop yield (bushels per acre).		
			Oats.	Wheat.	Barley.
		Feet.			
20.....	1/110	3×132	101. 58	42. 49	54. 50
20.....	1/40	8. 5×132	83. 70	27. 42	40. 70
20.....	1/10	34×132	79. 59	26. 92	.....

From the data given it is evident that shape as well as size of plot is of prime importance in considering increases in the yields of crops grown in plots surrounded by alleys. The larger the plots, provided the ratio of width to length remain approximately the same, and the nearer they approach the form of a square the smaller the percentage of total area exposed to alley effect and the less the increase in yield due to this cause.

However, comparatively long and narrow plots can be more easily sown with farm drills and harvested with binders than square plots of the same size. The removal of a sufficient number of border rows from the margins of long, narrow plots to obviate alley effect would appear to be the proper procedure. Leaving no alleys between plots at seeding time and removing a sufficient number of rows from each variety after full heading to provide a pathway of the desired width would accomplish similar results.

In Table IV are summarized the average yields, in bushels per acre, together with the standard deviations for four $\frac{1}{40}$-acre plots (approximate) of each of 11 varieties of oats, 5 varieties of wheat, and 4 varieties of barley (a) with no border rows removed, (b) with one border row on either side of each plot removed, and (c) with two border rows on either side of each plot removed. The yields included in this table are for only one season and are not intended as a variety test report. They are used only in so far as they supply data for the consideration of border effect in variety trials for one season. Comparing the average yields from the four $\frac{1}{40}$-acre plots of each variety, it is significant that in each instance the yields with no border rows removed are the highest, with one border row removed the next highest, and with two border rows removed, the lowest. Considering the average yields for all varieties, the oats, with no border rows removed, yielded 9.14 bushels; the wheat, 5.28 bushels; and the barley, 8.48 bushels per acre more than where two border rows were removed from either side of each plot. When only one border row was removed from either side of each plot, the oat varieties yielded 2.20 bushels, the wheat 1.99 bushels, and the barley 2.86 bushels higher than when two border rows were removed.

TABLE IV.—*Comparison of average yield, in bushels per acre, for four one-fortieth-acre plots (approximate size) with no border rows removed, with one border row removed from either side of each plot, and with two border rows removed from either side of each plot for 11 varieties of oats, 5 varieties of wheat, and 4 varieties of barley*

Crop and variety.	Descriptive note (time of maturity or type).	No border rows removed.			One border row removed.			Two border rows removed.		
		Yield (bushels per acre).	Rank.	Standard deviation.	Yield (bushels per acre).	Rank.	Standard deviation.	Yield (bushels per acre).	Rank.	Standard deviation.
OATS.										
Victory	Medium	99.42	1	4.69±1.12	88.90	1	4.80±1.15	88.57	1	3.50±0.83
Minota[a]	Medium early	87.11	2	2.47±0.59	77.00	2	1.54±0.37	77.06	2	1.13±0.27
Silver mine	Medium	85.68	3	3.45±0.82	78.00	3	2.91±0.69	75.64	3	3.20±0.76
Banner	Medium	82.16	4	5.89±1.40	74.46	4	5.96±1.42	72.82	5	5.90±1.41
Lincoln	Medium	80.71	5	5.65±1.35	72.77	7	5.14±1.23	70.01	7	5.37±1.28
O. A. C. 72	Medium	80.53	6	2.17±0.52	73.87	6	2.38±0.57	71.99	6	2.77±0.66
Iowa 103	Early	79.47	7	4.46±1.06	74.19	5	3.51±0.84	73.66	4	2.99±0.71
Swedish Select	Medium	75.80	8	4.51±1.08	69.23	8	3.46±0.83	66.55	8	3.84±0.92
Kherson	Early	73.40	9	1.59±0.44	67.47	9	1.50±0.41	65.10	9	2.48±0.68
White Tartar	Late	70.97	10	2.49±0.60	65.49	11	1.56±0.37	62.00	11	1.59±0.38
O. A. C. 3	Early	70.40	11	4.64±1.11	65.90	10	4.89±1.17	64.33	10	5.34±0.27
Average		80.51		3.82±	73.57		3.42±	71.37		3.47±
WHEAT.										
Marquis	*vulgare*	37.30	1	.58±1.39	33.41	1	.65±0.15	31.69	1	.31±0.07
Preston	*vulgare*	36.49	2	2.78±0.66	31.58	2	1.80±0.43	29.51	2	1.38±0.33
Glyndon	*vulgare*	30.82	3	3.55±0.85	27.43	3	3.70±0.88	25.74	4	3.47±0.83
Arnautka	Durum	29.59	4	2.70±0.64	27.60	4	2.05±0.50	25.94	3	2.27±0.54
Acme	Durum	28.44	5	3.59±0.86	26.20	5	3.09±0.74	24.39	5	3.01±0.72
Average		32.53		2.64±	29.24		2.46±	27.25		2.09±
BARLEY.										
Manchuria	Six-row	63.99	1	.59±0.14	47.60	2	.65±0.15	43.89	2	1.37±0.33
Chevalier	Two-row	52.92	2	1.50±0.36	48.15	1	1.77±0.42	46.33	1	1.31±0.31
Manchuria × Manchuria	Six-row	51.85	3	1.68±0.40	45.60	3	.69±0.16	42.81	3	.14±0.03
Manchuria × Manchuria	Six-row	47.72	4	.44±0.11	41.64	4	.23±0.05	38.45	4	.66±0.16
Average		51.37		1.05±	45.75		.84±	42.89		.87±

[a] Minota is a selection made at the Minnesota Experiment Station.

As shown in Table IV, the average standard deviations for the yields of the different varieties of oats, wheat, and barley in the tests with no border rows removed are somewhat greater in each instance than those for the tests with two border rows removed. This suggests the possibility that the probable error for a single determination of yields secured from a single variety grown in plots of a given size may in some instances be greater than the probable error of yields of the same plots after the removal of marginal areas 12 inches in width.

From the data submitted it seems fair to conclude that the extent of the increase in yield of wheat, oats, and barley grown in plots surrounded by alleys depends upon the size and shape of the plots and that the removal of the plants occupying an area at least 12 inches wide within the margins of plots removes alley effect and makes yields more nearly comparable.

ARE ALL VARIETIES AFFECTED ALIKE BY SURROUNDING ALLEYS?

If, when grown in plots surrounded by alleys, the yields of all varieties or of all cultural or fertilizer treatments are increased approximately alike, then, so far as the comparative results of the different varieties in any single test are concerned, the inclusion of the border rows of plots at harvest is not objectionable except in so far as has been noted. An opinion regarding this has been expressed by Barber (*1, p. 82*), as follows:

> All in all, as long as conditions are similar for all varieties of grain in trial in a field of fairly uniform soil, the results of plot tests will show the relative yields of the varieties.

Referring again to Table IV, it will be noted that in the column headed "No border rows removed" the varieties of oats, wheat, and barley are each listed in order of yield, in bushels per acre, for that method of test. Do the varieties maintain approximately the same relation with regards to yields when one and two border rows, respectively, are removed from either side of each plot?

An inspection of the rank of the different varieties, when one and two border rows, respectively, have been removed from either side of each plot shows some changes. If these changes in rank, due to the removal of border rows are fairly consistent throughout the 3- or 5-year period in tests conducted by the three methods, what effect will this have on the final interpretation of results?

The standard deviation for 20 control oat plots is 3.24 ± 0.35 bushels. On using this standard deviation in the formula

$$\frac{\text{Standard deviation} \times 0.6745}{\sqrt{n}}$$

in which n denotes the number of plots, to derive the probable error in bushels per acre for the yields obtained from plots replicated three times (four plots of each variety), the result is found to be 1.09 bushels (*11*). A difference of 4.07 between two results is necessary for odds of 40 to 1 against such a difference in one direction only being due to normal variation (*11*). Multiplying 4.07 by 1.09 bushels gives 4.44 bushels, which is the least difference between any two varieties, which is significant. This figure may be used in a broad way in considering the results for three methods of test. In the test with no border rows removed, Victory yielded 12.31 bushels more than any other variety. Since this difference is greater than 4.44 bushels, Victory oats may be considered the highest yielder under that method of test. Considering the other varieties in a similar way, Minota is not better than Silvermine, but is a higher yielder than Banner or any other variety in the trial yielding less than Banner. Silvermine is not better than Banner, but is superior to Lincoln or any other variety yielding less than Lincoln. Banner, Lincoln, O. A. C. 72 and Iowa 103 are higher yielders than Swedish Select, Kherson, White Tartar, and O. A. C. 3.

The differences in yield per acre for the various oat varieties brought about by the removal of one border row do not necessitate any material changes in the rank of the varieties as discussed for the test made without the removal of border rows.

By using 4.44 bushels as the least significant difference between any two varieties in the test of the oat varieties with two border rows removed, Victory still maintains the lead, but with less margin than in the test with no border rows removed. Minota can not be considered a higher yielder than Silvermine, Iowa 103, or Banner, but is superior to O. A. C. 72 and the other varieties lower than it in yield.

In the test with two border rows removed the variety Iowa 103 shows up as very promising, while in the test with no border rows removed it is of indifferent value.

The standard deviation for 20 control wheat plots is 2.45 ± 0.26. By using the standard deviation in deriving the probable error in bushels per acre for the yields of the wheat varieties as for the oats it is found to be 0.83 bushel. Multiplying 4.07 by 0.83 bushel gives 3.38 bushels as the least significant difference between any two varieties in the tests.

By using the 3.38-bushel difference in considering the varieties in the test with no border rows removed, it is clear that Marquis and Preston are not significantly different in yield, but are superior to the other varieties in the test. The removal of one or two border rows does bring about any significent changes in the rank of the wheat varieties.

The standard deviation for the 20 barley control plots is 2.53 ± 0.27. On deriving the probable error for the barley tests in the same way as the oats, the result is found to be 0.86 bushel. Multiplying 4.07 by 0.86 bushel gives 3.50 bushels as the least difference between any two barley varieties in the tests which may be considered significant.

In the test with no border rows removed the Manchuria cross, listed fourth, is significantly lower in yield than the three other varieties and retains that position in the tests with one and two border rows removed. The rank of Manchuria and Chevalier change in the test with one border row removed, but the difference between their yields is not significant.

In the test of the barley varieties with two border rows removed, the Chevalier yielded 2.44 bushels more than the Manchuria and 3.52 bushels more than the better of the two Manchuria crosses. For this method of test, the Chevalier is not significantly higher in yield than the Manchuria, but may be considered superior to the Manchuria cross. It seems that the barley varieties grown in plots surrounded by alleys are not equally efficient in utilizing the additional space.

The results indicate that, unless there is considerable fluctuation in the response of varieties to border effect when grown in plots surrounded by alleys, in a 3- or 5-year trial superior types of oats and barley may not be given their true rank unless at least two 6-inch drill rows within the margins of the plots are removed before harvest.

SUMMARY

(1) In plots made up of 6-inch drill rows of oats, wheat, and barley with 18-inch alleys between each two, the outside border rows yielded 83.5, 100.4, and 123.3 per cent, respectively, higher than the average from the central 13 rows. For oats, wheat, and barley the inside border rows (second drill rows within the margins of plots) yielded 23.23, 49.29, and 50.36 per cent, respectively, higher than the averages for the central 13 rows. Alley effect is operative over an area at least 12 inches wide within the margins of plots.

(2) The extent of increase due to alleys varies with the percentage of total area in at least a 12-inch strip within the margin of the plots. This percentage is greater for small plots as compared with larger ones of approximately the same shape and for long, narrow plots as compared with those more nearly approaching the form of a square.

(3) Plots 6 to 8 feet wide and 64 to 132 feet or more in length are more easily sown and harvested with the ordinary farm machinery than plots of the same size, but more nearly approaching the form of a square. Removal of the plants occupying an area at least 12 inches in width in comparatively long, narrow plots apparently obviates the most serious objection to their use in variety test work.

Oats, wheat, and barley, grown in plots 8.5 feet wide and 132 feet long after the end borders had been cut but with no side borders removed, yielded 9.14, 5.28, and 8.48 bushels more, respectively, than when two side border drill rows had been removed before harvest. With only one side border row removed from either side of each plot before harvest, the oat varieties yielded 2.20 bushels, the wheat 1.99 bushels, and the barley 2.86 bushels higher than when two drill rows had been removed.

(4) In a test of oat varieties in plots with two border rows removed, the rank in yield was not the same as when no border rows were discarded. The performance of one variety was very satisfactory by the former and indifferent by the latter method of test.

The indications are that the barley varieties grown in plots surrounded by alleys were not equally efficient in utilizing the additional adjacent space.

The removal of one or two side border drill rows in the wheat varieties did not bring about any significant changes in rank.

CONCLUSIONS

In plots surrounded by alleys plants occupying an area at least 1 foot within the margins are affected by the additional adjacent space.

The indications are that, unless there is a considerable fluctuation in the response of varieties to border effect, when grown in plots surrounded by alleys, superior types may not be given their true rank in tests made in plots from which borders are not removed before harvest.

These results have led to the adoption of the plan of removing the plants from an area at least one foot wide within the margins of variety test plots at the central and substations in Minnesota. These borders are to be removed from the plots between the time of fully heading and harvest.

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FUMIGATION OF CATTLEYA ORCHIDS WITH HYDROCYANIC-ACID GAS

By E. R. SASSCER, *Chief Inspector*, and H. F. DIETZ, *Entomological Inspector, Federal Horticultural Board, United States Department of Agriculture*

INTRODUCTION

As a preliminary, it will not be out of place to refer briefly to the regulations of the Federal Horticultural Board governing the importation of orchids prior to the adoption of fumigation with hydrocyanic-acid gas as a requirement for entry. These plants, which for the most part originate in countries that do not maintain a recognized inspection service, were allowed to enter under permit through designated ports of entry which were provided with Federal inspectors, or collaborators of the Federal Horticultural Board. Orchids arriving in the ports designated were examined by representatives of the board, and, if found to be free from insects and diseases believed to be injurious, were liberated from customs and allowed to proceed to the consignee.

INSECT PESTS INTERCEPTED

A total of 137 species of insects were collected on imported orchids, principally species of Cattleya, or in cases containing them, from August, 1912, to December 16, 1917, including 41 species of scale insects and a number of recently introduced ants which are now firmly established and responsible for considerable injury in greenhouses as far west as Indiana. A careful examination of three cases of Colombian orchids by the junior author revealed the presence of 17 species of insects distributed in the following orders: Orthoptera, Hymenoptera, Hemiptera, Coleoptera, Lepidoptera, Corrodentia, and Collembola.

Early in the spring of 1917 a shipment of 47 cases of cattleyas from Colombia was found, upon examination by Messrs. H. B. Shaw and D. G. Tower, of the Federal Horticultural Board, to be infested with larvæ of an unrecognized pyralid moth not known to occur in the United States. Inasmuch as these larvæ were present in numbers on and among the roots of the plants, the writers were instructed by the Federal Horticultural Board to conduct experiments immediately to determine the possibility of killing these larvæ and other insects by the vacuum process without removing the plants from the original container.

EXPERIMENTS TO DETERMINE THE POSSIBILITY OF KILLING INSECTS BY FUMIGATION WITHOUT REMOVING THE PLANTS FROM THE CONTAINER

To determine the effectiveness of this method of fumigating, the insects and plants were exposed to the gas under conditions comparable to those existing at commercial vacuum fumigation plants, where time would not permit the removal of the plants from the containers. To obtain this result, pyralid larvæ were fumigated as follows: Two in a vial plugged with cotton; two secreted in the roots of a cattleya wrapped in one thickness of newapaper; two secreted in the roots of a cattleya wrapped in cardboard and three thicknesses of newspaper; one in a vial plugged with cotton and wrapped with plant in two thicknesses of manila paper; two in nests of two and three pill boxes,[1] respectively.

The containers were placed in the fumigation chamber, the door closed, and a 20-inch vacuum produced. The gas was then generated, a dosage of 1 ounce of sodium cyanid [2] per 100 cubic feet of space being given, with an exposure of 40 minutes as follows: Five minutes were allowed for full generation and then 5 minutes to wash all the gas over to the fumigation chamber, after which the plants and insects were exposed to the gas for 30 minutes under normal atmospheric conditions. At the completion of the exposure the gas was removed from the fumigation chamber by producing a 25-inch vacuum. Upon examination all larvæ were found dead, irrespecive of their position, some being flattened out and void of viscera.

To determine further the penetration of hydrocyanic-acid gas under vacuum conditions, the following test was conducted with the black-walnut worm (*Datana integerrima* Grote and Robinson). This experiment differed slightly from the former in that the exposure was increased to one hour with a preliminary vacuum of 26 inches. The dosage remained the same, the larvæ being exposed to the gas under the following conditions: (1) Eight larvæ in a nest of triple pill boxes, inclosed in two tight-fitting cardboard coccid boxes,[3] the outer, or larger, box being wrapped with four thicknesses of dry newspaper and one layer of heavy wrapping paper and tied. This package was then placed in a wheat sack which was inclosed in a mail bag. (2) Four larvæ were similarly wrapped, except that the newspaper was thoroughly wet. (3) Four larvæ in triple pill boxes were inclosed in a tight-fitting screw-top mailing tube and placed in a thin seed sack within a wheat sack. (4) Same conditions as No. 3, except that the mailing tube was wrapped in six layers of wet newspaper. Upon completion of the exposure all larvæ were dead, whereas all larvæ similarly wrapped and held as controls were alive.

[1] Diameter of pill boxes as follows: 1¾, 1½, and 1¼ inches.

[2] Sodium cyanid guaranteed to contain not less than 51 per cent cyanogen and commercial sulphuric acid (about 1.84 sp. gr., or 66° Baumé) were used in all experiments referred to in this paper.

[3] These boxes measured 5 by 3 by 2 and 3 by 2 by 1½ inches.

EXPERIMENTS TO DETERMINE EFFECT OF FUMIGATION ON IMPORTED CATTLEYAS AT TIME OF ARRIVAL

A series of experiments under vacuum and normal atmospheric conditions was conducted to determine what effect, if any, the gas would have on the leaves, pseudobulbs, and general health of the plant. Mechanically injured and sound plants were purposely fumigated with excessive dosages to determine the appearance of fumigation injury. To induce burning, the leaves were slashed and bruised with a hatchet. Leaves so treated invariably exhibited blackened areas immediately around the injured spot, and yellowing was evident where the food supply was cut off. This yellowing, however, was no more pronounced in the fumigated plant than in the unfumigated ones, and was invariably confined to the old or injured leaves. The experience of the writers with the introductions of the Department of Agriculture has been that leaves on plants which have been poorly ventilated for a long period slowly turn yellow and eventually fall when exposed to the air. These leaves are apparently devitalized by abnormal shipping conditions, and the discoloration may be due to physiological changes.

To demonstrate that fumigation is not responsible for the shedding of all the leaves which are frequently found in containers when delivered to the consignee a number of cases were carefully examined at the port of entry. Two poorly ventilated cases yielded the following count: (1) Number of plants, 42; total number of leaves, 304, of which 234, or 76 per cent, had fallen from the plants and were in various stages of decay (Pl. 20). (2) Number of plants, 69; number of pseudobulbs on the plants, 719, of which 194 were dead; total number of leaves, 539, of which 257, or 47 per cent, were dead. These figures represent fairly well the condition of poorly packed orchids at the time of arrival, although there are several instances on record where the entire contents of the case were dead.

Orchids fumigated in New York on May 27, together with controls, were forwarded to Washington and held in a greenhouse. On July 30 both the fumigated and unfumigated plants had lost 33 per cent of their old leaves. As further evidence that fumigation with 1 ounce of sodium cyanid does not kill the plants, if they are in a reasonably good condition at the time of fumigation, a commercial orchid grower, on July 17, had cut 80 blooms from 700 plants of *Cattleya trianae* which were fumigated in New York during the latter part of May. It is apparent, therefore, that injury and shedding of leaves which might be attributed to fumigation at the port of entry are in a large measure due to poor packing, improper ventilation, and poor shipping conditions. This conclusion is further supported by the summarized experiments given in Tables I, II, and III.

TABLE I.—*Results of fumigation of cattleyas immediately upon arrival at Washington, D. C., under summer temperature conditions*

Dosage rate, in ounces of sodium cyanid.	Exposure.	Temperature.	Vacuum.	Conditions under which fumigated.	Number of leaves on—				Number of old leaves lost since fumigation.		Condition on Nov. 22, 1917.			Percentage of old leaves lost after fumigation.
					Date of fumigation, June 6, 1917.	June 25, 1917.	Nov. 22, 1917.		June 6–25.	June 25 to Nov. 22.	Buds or shoots.	Flowers.	Plants.	
							New.	Old.						
	Hrs.	°*F*	*In.*											
1.....	1	80	20	Thoroughly wet.	77 on 12 plants.	62	28	33	16	29	[a] 25	5	3 excellent, 7 good, 2 fair.	62
1.....	1	80	20	Dry.....	101 on 12 plants.	88	31	76	15	12	[b] 23	4	4 excellent, 4 good, 4 fair.	25
1.....	1	80	20	Thoroughly wet.	76 on 10 plants .	36	16	22	40	14	19	0	Poor before fumigation; 1 excellent, 2 good, 7 fair.	71
1.....	1	80	20	Dry.....	41 on 7 plants ..	20	12	15	21	5	[c] 8	0	Poor before fumigation; 3 good, 2 poor, 2 missing.	64
2.....	½	80	20	Wet.....	69 on 12 plants .	54	24	38	15	16	[d] 9	5	5 good, 5 poor, 2 missing.	70
2.....	½	80	20	Dry.....	65 on 13 plants .	30	16	27	35	3	[e] 21	0	1 excellent, 1 good, 1 poor, 2 missing.	88
4.....	⅔	80	25	.....do.....	61 on 4 plants..	32	14	20	29	12	2	2	Good, although 1 plant lost 15 pseudobulbs.	69
Control .	....	....	....	.....	35 on 9 plants ..	25	2	21	10	4	6	0	Good; pseudobulbs not counted.	40

[a] Ten growing. [b] Eleven growing. [c] One poor. [d] Four growing. [e] Fourteen growing.

TABLE II.—*Summary of experiments to determine penetration and effect of hydrocyanic-acid gas on cattleyas held unplanted for one month prior to fumigation. All box fumigations were conducted under normal atmospheric conditions*

Number of plants.	Treatment before, during, and after fumigation.	Rate in ounces of sodium cyanid.	Exposure.	Vacuum.	Temperature.	Relative humidity.	Number, when fumigated July 30, 1917, of—				Number, Nov. 22, 1917, of—						Percentage of old leaves lost after fumigation.
							Leaves.	Shoots.	Buds.	Pseudobulbs.	Leaves.		Pseudobulbs.		Buds.	Flowers.	
											Old.	New.	Old.	New.			
3..	Dry, wrapped 2 hours before fumigation and kept wrapped 24 hours after fumigation.....	1	*Hrs.* 1	*In.* 26	°*F.* 90	*Pct.* 60	13	5	0	[a] 32	11	1	29	1	6	1	15
4..	Same as above, except wet.....	1	1	26	90	60	10	3	1	31	6	5	29	6	2	0	40
3..	Same as above, except dry.....	1½	1	26	90	60	10	3	2	26	6	4	26	4	7	0	40
3..	Same as above, except wet.....	1½	1	26	90	60	11	1	3	[b] 37	9	4	27	4	7	0	18
3..	Dry, held in dark 2 hours prior to fumigation; not wrapped when fumigated.....	1½	1	(c)	90	60	11	2	0	23	11	8	18	8	4	0	0
1..	Wrapped in heavy paper 2 hours before fumigation and kept wrapped 24 hours.....	4	1	26	86	65	8	3	0	18	1	3	1	3	2	1	87
3..	Same as above, except wet	4	1	26	86	65	11	3	1	20	2	0	10	0	3	0	82
1..	Under canvas 2 hours before fumigation; not wrapped when fumigated	4	1	(c)	86	65	6	5	0	24	4	3	24	3	1	0	33½
4..	Control.....	....	....	....	....	....	17	8	0	32	14	3	28	3	4	2	17½

[a] Six in poor condition. [b] Eight scarred. [c] In box.

TABLE III.—*Results of experiments similar to those given in Table I, except that all plants fumigated were previously fumigated in New York City on May 27, 1917, with 1 ounce of sodium cyanid per 100 cubic feet, with an exposure of 40 minutes under partial vacuum as described in the text*

Number of plants.	Treatment before, during, and after fumigation.	Rate in ounces of sodium cyanid.	Exposure.	Vacuum.	Temperature.	Relative humidity.	Number, second fumigation, July 30, 1917, of—				Number, November 22, 1917, of—						Percentage of old leaves lost after fumigation.
							Leaves.	Shoots.	Buds.	Pseudobulbs.	Leaves.		Pseudo bulbs.		Buds.	Flowers.	
											Old.	New.	Old.	New.			
			Hr	*In.*	°*F*	*Pc*											
7	Wrapped 2 hours before fumigation and kept wrapped 24 hours	1	1	26	90	60	17	5	2	[a]56	11	4	49	10	10	1	35
7	Same as above, except wet	1	1	26	90	60	24	8	3	54	11	1	46	1	15	0	54
2	Same as above, except dry	1½	1	26	90	60	12	9	0	[b]48	1	6	45	5	9	0	91⅔
5	Same as above, except wet	1½	1	26	90	60	19	9	1	73	10	8	72	8	14	0	47
4	Wet, held in dark 2 hours prior to fumigation; not wrapped when fumigated	1½	1	(c)	90	60	16	8	0	37	13	7	34	6	4	0	18½
5	Control	....	....	....	....	....	28	8	0	46	23	8	41	7	7	1	17½

[a] Three in poor condition. [b] Five black. [c] In box.

All plants used in the experiments recorded in the tables were selected at random, effort being made in so far as possible to equalize the number of leaves and pseudobulbs in each test. Moreover, it was discovered after the plants bloomed that four species of Cattleya were represented—viz, *trianae*, *mossiae*, *schroederae*, and *percivaliana*. Unfamiliarity with these plants rendered it impossible for the writers to separate the different species by the characters of the pseudobulbs and leaves. So far as the different varieties were concerned, however, there was no noticeable difference in the final results. Of 116 plants fumigated, only 8, or approximately 7 per cent, died.

As indicated in the tables, the plants were fumigated under unfavorable conditions as regards temperature and moisture. This was deemed advisable, since orchids are offered for entry in large numbers during the summer months, and at this season it is frequently impossible to work with low temperatures; and, furthermore, these plants occasionally are drenched with water *en route*.

All plants listed in Table III received two fumigations, the first exposure being in New York on May 27, with sodium cyanid at the rate of 1 ounce per 100 cubic feet of space, with an exposure of 40 minutes and a preliminary 20-inch vacuum.

All plants used were grown by Dr. James S. Cannon, the orchid expert of the propagating gardens of the War Department, and in many cases were not potted for weeks after fumigation. It is evident, therefore, that the cattleyas were not given special treatment, but were handled as a commercial shipment.

The percentage of old leaves lost, as given in the tables, is neither distinctly correlated with conditions under which the plants were fumigated nor with the dosage given. Whether this discrepancy is due to specific resistance of the different species of Cattleya fumigated or to physiological conditions of the plants at the time of fumigation remains to be determined by the use of known plants kept under similar conditions before exposure to the gas. It will be noted, however, that plants which lost a high percentage of old leaves produced flowers, new leaves, and pseudobulbs. (Pl. 19.)

FUMIGATION AS A REQUIREMENT FOR ENTRY

Owing to the large number of insects which have been intercepted in cases of orchids and to extremely poor inspection facilities at the ports of entry, the Federal Horticultural Board has instructed its inspectors to require the fumigation of all orchids arriving in bulk from countries which do not maintain a recognized inspection service. On the strength of the experiments listed in this paper, all plants fumigated in the original container must be fumigated with 1 ounce of sodium cyanid, with an exposure of one hour. A preliminary vacuum of 20 inches is required prior to the generation of the gas.

CONCLUSIONS

(1) Black areas appear on unfumigated as well as fumigated leaves which have been injured.

(2) Progressive yellowing occurs on both unfumigated and fumigated plants and depends on adverse treatment or age of the leaves.

(3) Fumigated plants lose their leaves more rapidly than do unfumigated plants when subjected to adverse treatment. Young leaves and shoots are not severely injured by the gas with a 1-ounce dosage, although a number of old devitalized leaves may fall.

(4) Fumigation is not responsible for dying of pseudobulbs, if excessive dosages are not used.

(5) Presence of water on cattleya leaves does not increase burning from fumigation.

(6) Loss of a few old leaves does not render a plant valueless, as in a brief period they are replaced by new vigorous foliage.

(7) Where excessive dosages are not employed, orchids are apparently stimulated by hydrocyanic-acid gas. (See Pl. 19.)

(8) Infested orchids at the time of arrival at the port of entry, if in a reasonably good condition, are not seriously affected by hydrocyanic-acid gas generated at the rate of 1 ounce of sodium cyanid per 100 cubic feet, and are not killed where a 4-ounce dosage is used.

(9) Insects which are not hermetically sealed in stems or pseudobulbs of cattleyas can be killed in the original cases with hydrocyanic-acid gas, provided a preliminary 20-inch vacuum is given.

PLATE 19

Cattleya schroederae five months after having been fumigated with hydrocyanic-acid gas at the rate of 1 ounce per 100 cubic feet of space.

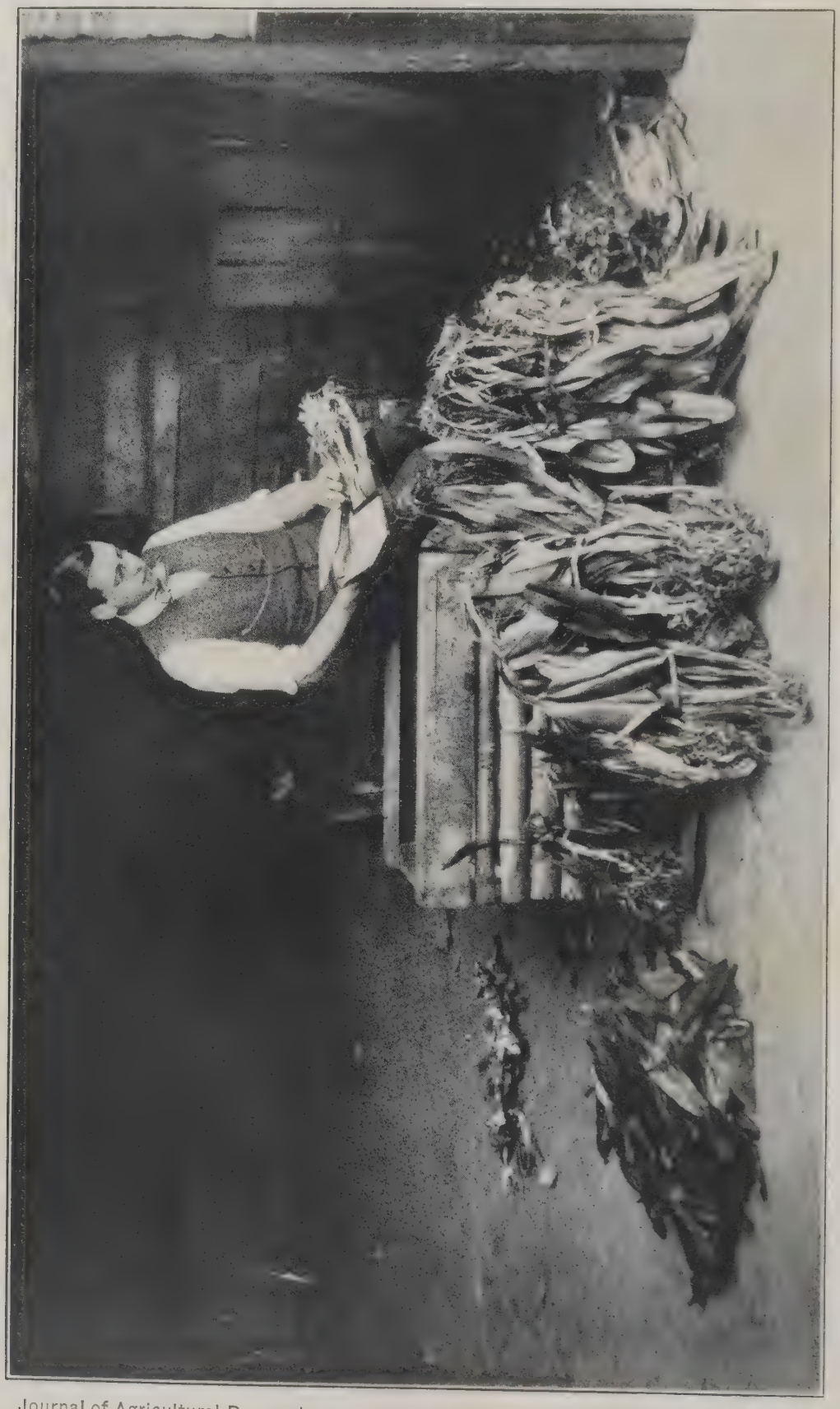

PLATE 20

Examining a case of cattleyas before fumigation at the port of New York. This case yielded the following count: Number of plants, 42; total number of leaves, 304; number of leaves off of the plants in various stages of decay, 234.

NET ENERGY VALUES OF ALFALFA HAY AND OF STARCH

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COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE

INTRODUCTION

The experiments here reported were undertaken primarily to determine the net energy value of starch as a representative of the carbohydrates. Alfalfa hay was used as the necessary roughage chiefly in order to secure a mixed ration not too low relatively in protein, but in part also for the sake of comparison with the results of several earlier determinations on the same feeding stuff.

OUTLINE OF EXPERIMENT

The subject of the experiment was a pure-bred Shorthorn steer, designated as Steer J, bred by The Pennsylvania State College. He was a year and 10 months old at the beginning of the experiment and had been chiefly grass fed up to that time. He was the same animal used in the subsequent year for the experiment on the influence of the degree of fatness of cattle upon their utilization of feed already reported.[1]

The general plan of the experiment was the same which has been employed in our more recent determinations of the net energy values of concentrates. It consisted, first, in determining with the aid of the respiration calorimeter, the net energy value of the roughage by a comparison of two or more periods in which different amounts of it were fed and second, in making similar determinations upon a mixture of hay and starch in the proportion of 2.5 to 1. By a difference calculation, precisely similar in principle to that commonly used in estimating the digestibility of a concentrate, the net energy of the starch could then be computed.[2] As a matter of fact, the trials were not actually made in this order, those on the mixed ration preceding those on the hay.

It may be noted that this method differs from that employed by Kellner, who has also reported a number of determinations of the net energy value of starch.[3] In his experiments the starch was added to a basal ration of hay and grain, the balance of carbon and nitrogen on each

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ration being determined with a respiration apparatus and the heat production computed. A difference calculation, after making certain corrections, shows the net energy of the added starch.

PERIOD AND RATIONS

Each feeding period covered three weeks, of which the first 11 days constituted the preliminary period while during the last 10 the feces and urine were collected quantitatively. A 48-hour determination of the respiratory products and of the heat production was made on the eighteenth and nineteenth days of each period. The dates of the several periods, the rations fed and the average live weight of the animal in each period are shown in Table I.

TABLE I.—*Periods, rations, and average live weights*

Period.	Preliminary period.	Digestion period.	Daily rations.		Live weights of steer.
			Hay.	Starch.	
	1912–13.	1913.	*Kgm.*	*Kgm.*	*Kgm.*
Period 1	Dec. 22–Jan. 1	Jan. 2–Jan. 11	7.50	3.00	388.7
Period 2	Jan. 12–Jan. 22	Jan. 23–Feb. 1	3.00	1.20	366.5
Period 3	Feb. 2–Feb. 12	Feb. 13–Feb. 22	4.25	1.70	387.0
Period 4	Feb. 23–Mar. 5	Mar. 6–Mar. 15	2.00	.80	356.2
Period 5	Mar. 16–Mar. 26	Mar. 27–Apr. 5	9.00	.....	403.8
Period 6	Apr. 6–Apr. 16	Apr. 17–Apr. 26	7.00	.....	403.2
Period 7	Apr. 27–May 7	May 8–May 17	4.00	.....	377.0

COMPOSITION OF FEEDING STUFFS

The alfalfa hay was from a car of baled hay purchased the previous year in Kansas City, Mo., and stated to have been grown in Idaho. The starch was commercial cornstarch

TABLE II.—*Composition and energy content of dry matter of feeding stuffs*

	Alfalfa hay.					Starch, Periods 1–4.
	Period 1.	Periods 2 and 3.	Periods 4 and 5.	Periods 6 and 7.	Average.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ash	9.74	8.83	9.27	9.43	9.26	0.24
Protein	11.34	10.96	11.36	11.51	11.29	.35
Nonprotein	3.27	3.21	3.03	3.12	3.14	.....
Crude fiber	30.69	31.86	29.46	29.96	30.47	.13
Nitrogen-free extract	43.22	43.38	45.14	44.21	44.10	99.28
Ether extract	1.84	1.76	1.74	1.77	1.77	.....
	100.00	100.00	100.00	100.00	100.00	100.00
Total nitrogen	2.51	2.44	2.46	2.51	2.48	.06
Protein nitrogen	1.82	1.75	1.82	1.84	1.81	.....
Carbon	45.00	44.94	44.98	44.74	44.92	43.64
Energy, calories per kgm.	4,335.34	4,389.90	4,332.82	4,334.67	4,348.18	4,104.85

Four samples of the finely cut alfalfa hay and four of the starch were taken as the rations were being weighed out. The hay samples were analyzed separately, while the starch samples were united to form a single composite. The analytical results and the periods covered by each sample are shown in Table II.

DIGESTIBILITY

The digestibility of the total ration was determined in the usual manner. The results are recorded in full in Table 1 of the Appendix and are summarized in Table III. The weighted means are computed from the totals of each ingredient eaten and excreted respectively in all the periods averaged. The digestibility of the starch is computed on the assumption that the hay fed with it had the digestibility shown by the mean of periods 5, 6 and 7. Large negative coefficients for protein and crude fiber result from this computation. The results for the other ingredients are as shown in the table, the periods being, for convenience of reference, grouped by the nature of the ration and arranged in each group in the order of magnitude of the rations.

TABLE III.—*Summary of percentage digestibility*

Feed and period.	Dry matter.	Ash.	Organic matter.	Protein.	Nonprotein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Total nitrogen.	Carbon.	Energy.
ALFALFA HAY											
Period 5, 7,893 gm.....	58.22	41.04	59.98	66.86	87.15	41.24	70.35	15.96	72.57	56.94	56.73
Period 6, 6,128 gm.....	59.49	40.92	61.42	69.21	89.52	44.07	71.27	8.96	72.64	57.59	57.55
Period 7, 3,502 gm.....	60.47	42.31	62.36	69.54	90.65	44.71	72.04	22.49	73.55	58.64	58.65
Weighted means..	59.11	41.25	60.96	68.23	88.66	42.94	71.00	14.81	72.79	57.50	57.40
ALFALFA HAY AND STARCH											
Period 1, 8,821 gm.....	68.86	44.54	70.68	60.04	87.64	39.26	83.11	23.57	62.99	67.64	67.30
Period 3, 5,299 gm.....	67.73	48.86	69.00	56.13	88.86	41.85	81.20	21.18	62.80	66.04	65.98
Period 2, 3,750 gm.....	70.12	47.42	71.65	61.92	88.57	44.96	83.43	17.81	66.31	68.74	68.57
Period 4, 2,480 gm.....	70.61	48.63	72.17	63.13	88.70	42.80	84.09	11.15	64.68	68.95	68.48
Weighted means..	69.01	46.62	70.61	59.77	88.25	41.49	82.78	20.45	63.71	67.58	67.34
Weighted means, omitting period 3.....	69.46	45.88	71.17	61.01	88.04	41.35	83.36	20.20	64.07	68.13	67.82
STARCH (Computed using mean digestibility of hay)											
Period 1.....	92.62	.....	92.11	.....	.....	.....	96.46	.....	.....	93.10	92.78
Period 3.....	88.55	.....	86.76	.....	.....	.....	91.96	.....	.....	87.28	88.14
Period 2.....	96.48	.....	95.03	.....	.....	.....	96.45	.....	.....	96.45	97.16
Period 4.....	98.39	.....	96.80	.....	.....	.....	98.46	.....	.....	97.48	96.75
Weightedmeans..	92.98	.....	91.83	.....	.....	.....	95.53	.....	.....	92.74	92.87
Weighted means, omitting period 3.....	94.55	.....	93.61	.....	.....	.....	96.80	.....	.....	94.66	94.53

The negative coefficients obtained for the protein and crude fiber of the starch by the foregoing method of computation are, of course, sinply an expression of the well-known fact that the addition of starch to a ration

usually results in depressing the digestibility of these ingredients. If it be assumed that the starch was completely digestible—that is, if all the effects just mentioned be assigned to the hay—the digestibility of the latter as compared with that observed in the periods when the hay was fed alone was as shown in Table IV.

TABLE IV.—*Influence of starch on digestibility of hay*

	Dry matter.	Ash.	Organic matter.	Protein.	Nonprotein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Total nitrogen.	Carbon.	Energy.
Computed digestibility, assuming 100 per cent for starch:	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Period 1	56.08	43.96	57.38	59.54	87.64	39.15	67.80	23.57	62.64	54.75	54.59
Period 3	50.83	48.28	54.96	55.55	88.86	41.76	63.37	21.18	62.43	52.38	52.82
Period 2	57.64	46.81	58.69	61.42	88.57	44.77	67.60	17.81	66.01	56.06	56.30
Period 4	58.44	48.07	59.50	62.67	88.70	42.70	69.60	11.15	64.35	56.48	56.12
Weighted means	56.21	46.03	57.25	59.25	88.25	41.39	66.86	20.45	63.40	54.54	54.62
Weighted means, omitting period 3	56.86	45.29	58.06	60.51	88.04	41.25	68.05	20.20	63.73	55.36	55.27
Observed digestibility	59.11	41.25	60.96	68.23	88.66	42.94	71.00	14.81	72.79	57.50	57.40
Difference	−2.25	+4.04	−2.90	−7.72	−0.62	−1.67	−2.95	+5.39	−9.06	−2.14	−2.13

In some respects a more rational method of expressing this depression in digestibility is in terms of the actual amounts instead of the percentages of the various ingredients. Table V shows the number of grams by which the several nutrients actually digested from the mixed ration of periods 1 to 4 differ from what would be expected on the same assumption as in Table IV.

TABLE V.—*Depression of digestibility by starch*

Period No.	Protein.	Nonprotein.	Crude fiber.	Total nitrogen.
	Gm.	*Gm.*	*Gm.*	*Gm.*
1	61.7	2.1	68.6	16.0
3	52.1	−.2	14.1	9.5
2	19.7	.1	−16.3	4.4
4	11.1	.0	1.2	3.6

INFLUENCE OF QUANTITY OF FEED ON DIGESTIBILITY

The figures of Table III show a distinct influence of the quantity of feed upon the percentage digestibility, as has been the case in most previous experiments.[1] The digestibility increases as the amount consumed is decreased, although the differences are relatively small. To this rule period 3 constitutes a distinct exception. As will appear, the results of

[1] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 613–618. New York, 1917.
——— and FRIES, J. A. ENERGY VALUES OF HOMINY FEED AND MAIZE MEAL FOR CATTLE. *In* Jour. Agr. Research, v. 10, no. 12, p. 605. 1917.
——— ———. INFLUENCE OF THE DEGREE OF FATNESS OF CATTLE UPON THEIR UTILIZATION OF FEED. *In* Jour. Agr. Research, v. 11, no. 10, p. 453. 1917.
——— ——— and BRAMAN, W. W. ENERGY VALUES OF RED-CLOVER HAY AND MAIZE MEAL. *In* Jour. Agr. Research, v. 7, no. 9, p. 381. 1916.

this period appear somewhat exceptional in other respects also, and the mean digestibility, excluding period 3, is therefore included in the table.

URINARY EXCRETION

From the results for the urinary excretion contained in Table 2 of the Appendix the following averages are derived. Table VI shows both the energy of the urine as actually determined and also with a correction of 7.45 Calories per gram for the gain or loss of nitrogen by the body.

TABLE VI.—*Average daily excretion in urine*

Feed and period.	Nitrogen.	Carbon.	Energy.		Ratio of nitrogen to carbon.	Observed energy.	
			Observed.	Corrected to nitrogen equilibrium.		Per gram of nitrogen.	Per gram of carbon.
ALFALFA HAY.							
	Gm.	*Gm.*	*Cal.*	*Cal.*		*Cal.*	*Cal.*
Period 5	129.7	196.4	1,916.1	1,992.1	1:1.51	14.77	9.76
Period 6	100.9	151.5	1,489.5	1,560.3	1:1.50	14.76	9.83
Period 7	70.6	107.7	997.5	943.9	1:1.53	14.13	9.26
ALFALFA HAY AND STARCH							
Period 1	66.8	140.5	1,229.0	1,467.4	1:2.10	18.40	8.75
Period 3	49.7	100.5	902.6	955.5	1:2.02	18.16	8.98
Period 2	43.1	78.3	733.3	725.1	1:1.82	17.01	9.37
Period 4	35.1	58.8	564.9	505.3	1:1.68	16.09	9.61

GASEOUS EXCRETION

The excretion of water vapor, carbon dioxid, and methane for each day is recorded in Table 4 of the Appendix. The average results for each period were as follows:

TABLE VII.—*Average daily gaseous excretion*

Feed and period.	Water.	Carbon dioxid.	In combustible gases.		Methane computed from carbon.	Ratio of hydrogen to carbon.
			Hydrogen.	Carbon.		
ALFALFA HAY.						
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Period 5	6,995.3	5,505.0	42.4	129.6	173.2	3.057
Period 6	5,458.7	4,568.6	33.5	99.3	132.7	2.970
Period 7	3,460.1	3,252.5	26.1	59.3	79.2	2.267
ALFALFA HAY AND STARCH.						
Period 1	10,028.1	7,045.5	54.9	168.5	225.1	3.069
Period 3	5,252.6	4,724.9	37.7	116.7	155.9	3.092
Period 2	4,384.3	3,909.5	27.1	83.8	112.0	3.092
Period 4	3,168.8	3,133.7	19.8	62.4	83.3	3.145

GAINS BY BODY

From the data of the Appendix the gains of protein, fat, and water by the animal can be computed in the usual way with the results there recorded in Table 6. In this computation the amount of water produced by the oxidation of organic hydrogen has been disregarded. The results are used only as a correction in the manner described on page 277, and it may be shown that a disregard of the organic hydrogen introduces no material error. Table VIII contains a summary of the gains of protein and fat by periods.

TABLE VIII.—*Average daily gains of protein and fat*

Feed and period.	Protein.	Fat.
ALFALFA HAY.		
	Gm.	*Gm.*
Period 5	61.2	205.3
Period 6	57.0	60.9
Period 7	−43.2	−154.0
ALFALFA HAY AND STARCH.		
Period 1	192.0	422.9
Period 3	42.6	34.1
Period 2	− 6.6	−106.2
Period 4	−48.0	−252.6

METHANE FERMENTATION

The extent of the methane fermentation per kilogram of dry matter of the feed and per 100 gm. of digested carbohydrates is shown in Table IX. In the hay the carbohydrates are the sum of the digested crude fiber and nitrogen-free extract, while in the case of starch the comparison is made with the digested nitrogen-free extract. The minute amount of crude fiber contained in the starch, as already noted, showed an apparent negative digestibility.

The extent to which the carbohydrates of the hay were subject to the methane fermentation was substantially the same as the average of 17 previous determinations on alfalfa[1]—viz, 4.9 gm. of CH_4 per 100 gm. of carbohydrates as compared with an average of 4.8. With the mixed ration of hay and starch the fermentation showed a distinct increase as the amount of feed was diminished. When, as in the latter part of the table, this increase is computed upon the starch alone, assuming average figures for the hay, it becomes, of course, relatively more marked and considerably exceeds Kellner's average of 3.07.[2] The relative extent of the fermentation was apparently abnormally great in period 3 which, as already noted, also gave apparently exceptional results as regards digestibility. The means, omitting period 3, have therefore been included in the table.

[1] ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. *In* Jour. Agr. Research, v. 3, no. 6, p. 450. 1915.

[2] KELLNER, O., and KÖHLER, A. UNTERSUCHUNGEN ÜBER DEN STOFF- UND ENERGIE-UMSATZ DES ERWACHSENEN RINDES BEI ERHALTUNGS- UND PRODUKTIONSFUTTER. *In* Landw. Vers. Stat., Bd. 52, p. 223. 1900.

TABLE IX.—*Methane production*

Feed and period.	Dry matter eaten.	Methane.		
		Total.	Per kilogram of dry matter.	Per 100 grams of digested carbohydrates.
ALFALFA HAY.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Period 5	7,893.0	173.19	21.94	5.0
Period 6	6,127.8	132.74	21.66	4.8
Period 7	3,501.6	79.19	22.61	5.0
Totals and means	17,522.4	385.12	21.98	4.9
ALFALFA HAY AND STARCH.				
Period 1	8,821.0	225.14	25.52	4.4
Period 3	5,298.8	155.93	29.42	5.1
Period 2	3,750.4	112.00	29.86	5.0
Period 4	2,480.0	83.34	33.60	5.6
Totals and means	20,350.2	576.41	28.32	4.8
Totals and means, omitting period 3	15,051.4	420.48	27.94	4.7
STARCH, COMPUTED, WITH MEAN FOR HAY.				
Period 1, total	8,821.0	225.14		
Period 1, hay	6,254.7	137.48		
Period 1, starch	2,566.3	87.66	34.16	3.6
Period 3, total	5,298.8	155.93		
Period 3, hay	3,747.7	82.37		
Period 3, starch	1,551.1	73.56	47.42	5.2
Period 2, total	3,750.4	112.00		
Period 2, hay	2,645.4	58.15		
Period 2, starch	1,105.0	53.85	48.73	5.1
Period 4, total	2,480.0	83.34		
Period 4, hay	1,754.0	38.55		
Period 4, starch	726.0	44.79	61.70	6.3
Totals and means	5,948.4	259.86	43.69	4.6
Totals and means, omitting period 3	4,397.3	186.30	42.37	4.4

METABOLIZABLE ENERGY

By metabolizable energy is understood the total chemical energy of the feed, as measured by its heat of combustion, minus the chemical energy lost in the feces, urine, and combustible gases. In other words, it is the amount of energy capable of conversion into other forms in the body. Correcting the observed energy of the urine to a state of nitrogen equilibrium[1] the following results (Table X) may be computed as in the case of digestibility. Averages have also been computed omitting the results of period 3.

[1] By adding 7.45 Calories for each gram of nitrogen retained by the animal or subtracting the same amount for each gram of body nitrogen lost, the correction being regarded as representing energy of excretory material temporarily retained in the body.

TABLE X.—*Losses of chemical energy; metabolizable energy*

Feed and period.	Energy per kilogram of dry matter.					Metabolizable energy per kilogram digestible organic matter.	Percentage losses.			Percentage metabolizable.
	Total.	Losses.			Metabolizable.		In feces.	In urine.	In methane.	
		In feces.	In urine.[a]	In methane.						
ALFALFA HAY.	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*				
Period 5	4,333	1,875	252	293	1,913	3,516	43.27	5.82	6.76	44.15
Period 6	4,335	1,840	255	289	1,951	3,507	42.45	5.87	6.67	45.01
Period 7	4,335	1,792	270	302	1,971	3,490	41.35	6.22	6.96	45.47
Weighted averages.	4,334	1,846	257	293	1,938	3,507	42.60	5.92	6.77	44.71
ALFALFA HAY AND STARCH.										
Period 1	4,267	1,395	166	341	2,365	3,596	32.70	3.90	7.98	55.42
Period 3	4,306	1,465	180	393	2,268	3,509	34.02	4.20	9.12	52.66
Period 2	4,306	1,353	193	398	2,362	3,516	31.43	4.49	9.26	54.82
Period 4	4,266	1,345	204	448	2,269	3,368	31.51	4.78	10.51	53.20
Weighted averages.	4,284	1,400	180	378	2,326	3,530	32.68	4.20	8.82	54.30
Weighted averages for periods 1, 2, and 4	4,276	1,376	179	373	2,348	3,538	32.18	4.19	8.72	54.91
COMPUTED FOR STARCH.										
Period 1	4,105	296	−53	456	3,406	3,707	7.22	−1.30	11.10	82.98
Period 3	4,105	487	−12	623	3,007	3,474	11.86	−0.29	15.19	73.24
Period 2	4,105	116	34	541	3,314	3,495	2.84	0.83	15.62	80.71
Period 4	4,105	134	76	823	3,072	3,182	3.25	1.86	20.05	74.84
Weighted averages.	4,105	293	−11	579	3,244	3,541	7.14	−0.24	14.10	79.03
Weighted averages for periods 1, 2, and 4	4,105	224	−10	563	3,328	3,563	5.46	−0.24	13.71	81.07
Kellner's average	4,152	731	−27	382	3,066	3,603	17.61	−0.66	9.21	73.84

[a] Corrected to N equilibrium.

It has already been noted that with the exception of period 3 the digestibility increased as the total amount of the ration was decreased. The converse of this is shown in Table X, of course, in the decreased losses in the feces. On the other hand, the losses in the urine and methane show a distinct increase on the lower rations. As in previous experiments, the greater digestibility of the smaller rations was apparently due largely to the greater extent of the methane fermentation accompanied by the excretion of more katabolic products of some sort in the urine. As a consequence, the metabolizable energy per kilogram of digested dry matter

or of digested organic matter was somewhat less on the lighter rations despite their higher digestibility. The difference would be still more marked if account were taken of the fact that on the lighter rations a larger proportion of the metabolizable energy is accounted for by the heat of fermentation of the carbohydrates.

The metabolizable energy per kilogram of digested organic matter agrees closely with the results obtained by us [1] in earlier experiments on roughage and by Kellner [2] in his experiments on starch.

HEAT EMISSION AND PRODUCTION

The results of the measurements of heat emission are contained in Table 5 of the Appendix. The heat emission, however, does not usually correspond with the heat production.

First, any matter gained by the body is raised from the temperature of the calorimeter to the temperature of the body and a corresponding amount of the heat produced is stored up as heat in this added material.

Second, a rise in body temperature while the animal is in the calorimeter likewise absorbs a certain amount of the heat actually produced. Both these amounts must therefore be added to the heat emission to get the real heat production. Of course this correction may be negative—that is, a fall of body temperature or a loss of body weight gives a negative correction—so that in such a case the heat production is less than the heat emission. The corrections due to these two causes are shown in the Appendix, Tables 6 and 7, while Table 8 shows the heat emission, the corrections, the heat production as thus computed and also the heat production as computed in the usual way from the balance of nitrogen and carbon—that is, by indirect calorimetry.

CORRECTION FOR STANDING AND LYING

Standing as compared with lying exerts such a marked influence upon the metabolism of cattle that it is necessary to make a correction for this factor in order to render the different periods of an experiment comparable. The heat production has therefore been computed to 12 hours each standing and lying in the manner described in a previous paper.[3] On using the same corrections as before for body gain and body temperature the results are as shown in Table XI.

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[1] ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. *In* Jour. Agr. Research, v. 3, no. 6, p. 451. 1915.

——— ——— ENERGY VALUES OF HOMINY FEED AND MAIZE MEAL FOR CATTLE. *In* Jour. Agr. Research, v. 10, no. 12, p. 605. 1917.

——— ——— and BRAMANN, W. W. ENERGY VALUES OF RED-CLOVER HAY AND MAIZE MEAL. *In* Jour. Agr. Research, v. 7, no. 9, p. 382.

[2] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 301. New York, 1917.

——— and FRIES, J. A. OP. CIT., 1915, p. 452.

[3] ARMSBY, H. P., and FRIES, J. A. OP. CIT., 1915, p. 454.

TABLE XI.—*Daily heat production computed to 12 hours' standing and lying.*

Period and ration.	First day.	Second day.	Mean.
ALFALFA HAY.	*Calories.*	*Calories.*	*Calories.*
Period 5	13, 309	13, 187	13, 248
Period 6	10, 940	11, 512	11, 226
Period 7	8, 981	8, 747	8, 864
ALFALFA HAY AND STARCH.			
Period 1	16, 394	15, 635	16, 015
Period 3	11, 283	11, 439	11, 361
Period 2	10, 150	9, 689	9, 920
Period 4	8, 483	8, 325	8, 404

ENERGY EXPENDITURE CONSEQUENT UPON FEED CONSUMPTION

The heat production in this experiment shows the same marked dependence upon the amount of feed consumed which has been noted in all previous investigations. By a difference computation, the heat increment caused by a unit of feed consumed may be computed. For example, a comparison of periods 5 and 7 gives the following results:

TABLE XII.—*Example of computation of heat increment due to feed*

Period.	Dry matter eaten.	Heat production.
	Gm.	*Calories.*
Period 5	7, 893	13, 248
Period 7	3, 502	8, 864
Difference	4, 391	4, 384
Difference per kilogram of dry matter	.....	999

TABLE XIII.—*Heat increment per kilogram of dry matter*

Feed and period.	Heat production per kilo.	Feed and period.	Heat production per kilo.
ALFALFA HAY.	*Cal.*	ALFALFA HAY AND STARCH—CON.	*Cal.*
Periods 5–6	1, 146	Periods 1–4	1, 200
Periods 6–7	899		
Periods 5–7	999	STARCH.	
ALFALFA HAY AND STARCH.		Periods 1–3	2, 118
		Periods 3–2	762
Periods 1–3	1, 321	Periods 2–4	1, 649
Periods 3–2	931	Periods 1–2	1, 704
Periods 2–4	1, 192	Periods 1–4	1, 692
Periods 1–2	1, 202		

In the same manner the heat increment per kilogram of the mixed ration of hay and starch may be computed, while by a comparison of these results with those on hay the energy expenditure per kilogram of

starch may be calculated. Three comparisons are obviously possible on the hay and five on the mixed ration. The results are contained in Table XIII.

It may be presumed that, other things being equal, the results obtained by comparing the extreme periods—namely, periods 5 and 7 for hay and periods 1 and 4 for the mixed ration—will be the most accurate. In the case of the hay the intermediate comparisons show differences similar to those already reported.[a] In the case of the mixed ration and of the starch the results show a marked degree of uniformity with the exception of those involving period 3. As already noted, the results of this period as regards digestibility, methane production, and metabolizable energy appear somewhat abnormal as compared with those of periods 1, 2, and 4, the losses in feces and methane being relatively large and the percentage of the gross energy which was metabolizable being correspondingly lower. This might be expected to result in a lower heat production. If such were the fact, it would tend to explain the marked divergence of those heat increments in Table XIII which involve the use of period 3. We are inclined therefore to accept the results of the extreme periods—viz,

Hay ..... 999 Calories per kilogram
Hay and starch ..... 1,200 Calories per kilogram
Starch ..... 1,692 Calories per kilogram

as representing most accurately the heat increment caused by the consumption of these materials.

NET ENERGY VALUES

Subtracting from the gross energy the losses of chemical energy and the heat increment due to feed consumption gives the net energy value as follows (Table XIV):

TABLE XIV.—*Net energy values per kilogram of dry matter*

Feed and period.	Gross energy.	Losses of chemical energy.	Metabolizable energy.	Heat increment.	Net energy values.	Net metabolizable.
ALFALFA HAY.	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Per ct.*
Average	4,334	2,396	1,938	[b] 999	939	48.45
ALFALFA HAY AND STARCH.						
Average of all	4,284	1,958	2,326	[c] 1,200	1,126	48.41
Average of periods 1, 2, and 4	4,276	1,928	2,348	[c] 1,200	1,148	48.89
STARCH.						
Average of all	4,105	861	3,244	[c] 1,692	1,552	47.84
Average of periods 1, 2, and 4	4,105	777	3,328	[c] 1,692	1,636	49.16
Kellner's average	4,152	1,101	3,051	1,248	1,803	58.80

[a] ARMSBY, H. P., and FRIES, J. A. OP. CIT., 1915, p. 473–475.
[b] From results of periods 5 and 7.
[c] From results of periods 1 and 4.

COMPARISON WITH KELLNER'S RESULTS ON STARCH

As noted at the beginning of this article, Kellner has reported a number of determinations of the net energy of starch. His results, expressed in terms of energy and computed in a somewhat different manner than that used by Kellner himself,[1] are included in Table XIV. Two marked differences appear. Kellner's metabolizable energy is lower than that found in our experiment, while his heat increment is also less, the net result being a higher net energy value. Kellner's rations, however, differ quite materially from ours. As regards quantity (total organic matter) our mixed ration was considerably above Kellner's in period 1 and much below it in periods 2 and 4. As regards make-up it contained a much larger proportion of hay and no concentrate except starch. The percentage of starch in the total ration was 31.2 as against 19.2 in Kellner's, but the percentage of organic matter supplied by the hay was 69.8 as compared with 40.9 in Kellner's experiments. As regards nutritive ratio our ration was somewhat narrower than three of Kellner's rations and somewhat wider than the other two.

The difference in the metabolizable energy is due chiefly to a much larger loss in the feces in Kellner's experiments, as Table X shows. If this be eliminated, by computing the losses in urine and methane upon the digested energy the following comparisons are obtained:

TABLE XV.—*Distribution of digested energy of starch*

	In urine.	In methane	Metabolizable.
	Per cent.	*Per cent.*	*Per cent.*
Mean of periods 1, 2, and 4	—0.26	14.50	85.76
Kellner's mean	— .79	11.17	89.62

The higher digestibility of the starch in our experiment was accompanied (caused?) by a greater production of methane, so that a less proportion of the digested energy was metabolizable. When thus computed on the digested energy, our results in period 1 agree substantially with Kellner's, while periods 2 and 4 show a considerably greater relative loss of energy in the methane. Period 3, as already noted, appears exceptional. No obvious explanation presents itself for the high heat increment found in our experiment. The more extensive fermentation of the starch may perhaps account for a portion, but by no means all of it.

[1] ARMSBY, H. P. OP. CIT., 1917, p. 455–459, 474.

SUMMARY

In seven respiration calorimeter experiments on a steer the digestibility and metabolizable energy of different amounts of alfalfa hay and of a mixture of alfalfa hay and commercial starch the gaseous excretion and the heat production were determined.

By a comparison of periods in which different amounts of the same ration were fed, the heat increments consequent on feed consumption and the net energy values of the rations were computed.

The digestibility of the rations, the losses in the urine, and the extent of the methane fermentation showed a distinct increase as the total amount of the ration was reduced.

The greater loss of energy in the urine and methane on the lighter rations more than compensated for the smaller losses in the feces, so that the proportion of the total energy metabolizable was somewhat less than on the heavier rations.

The metabolizable energy of the starch was 10 per cent greater than the average computed from five experiments by Kellner, the difference being due chiefly to smaller losses in the feces. Starch caused the usual depresion in digestibility.

The average heat increment caused by the consumption of alfalfa hay was 999 Calories per kilogram of dry matter as compared with 981 Calories found for the same hay in the previous year and with an average of 1,169 Calories in six previous experiments on three different samples.

The average heat increment for the starch was 1,692 Calories per kilogram of dry matter as compared with 1,248 Calories computed from Kellner's experiments.

The net energy values of the starch was about 9 per cent lower than that computed from Kellner's experiments, only 49 per cent, as compared with 59 per cent of the metabolizable energy being utilized by the animal.

APPENDIX

The principal numerical data obtained in the experuments are recorded in the following tables. The computations involved have been carried out beyond the probable limit of accuracy of the experimental methods in order to guard against a possible accumulation of arithmetical errors.

TABLE 1.—*Digestibility of rations*

Feed and period.	Dry matter.	Ash.	Organic matter.	Protein.	Non-protein.	Crude fiber.	N-free extract	Ethe extract.	Total nitrogen.	Carbon	Energy.
ALFALFA HAY.											
Period 5:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Calories.*
Total fed	7,893.0	732.0	7,161.0	896.9	238.9	2,325.5	3,562.5	137.2	194.3	3,550.3	34,198.95
Feces	3,297.6	431.6	2,866.0	297.2	30.7	1,366.5	1,056.3	115.3	53.3	1,528.7	14,796.30
Amount digested	4,595.4	300.4	4,295.0	599.7	208.2	959.0	2,506.2	21.9	141.0	2,021.6	19,402.65
Percentage digestibility	58.22	41.04	59.98	66.86	87.15	41.24	70.35	15.96	72.57	56.94	56.73
Period 6:											
Total fed	6,127.8	577.9	5,549.9	705.5	190.9	1,836.1	2,709.1	108.2	153.5	2,741.3	26,561.99
Feces	2,482.4	341.4	2,141.0	217.2	20.2	1,026.9	778.3	98.5	42.0	1,162.7	11,276.20
Amount digested	3,645.4	236.5	3,408.9	488.3	170.7	809.2	1,930.8	9.7	111.5	7,578.6	15,285.79
Percentage digestibility	59.49	40.92	61.42	69.21	89.52	44.07	71.27	8.96	72.64	57.59	57.55
Period 7:											
Total fed	3,501.6	330.2	3,171.4	403.1	109.1	1,049.2	1,548.1	61.8	87.7	1,566.4	15,178.28
Feces	1,384.2	190.5	1,193.7	122.8	10.2	580.1	432.8	47.9	23.2	647.9	6,276.31
Amount digested	2,117.4	139.7	1,977.7	280.3	98.9	469.1	1,115.3	13.9	64.5	918.5	8,901.97
Percentage digestibility	60.47	42.31	62.36	69.54	90.65	44.71	72.04	22.49	73.55	58.65	58.65
ALFALFA HAY AND STARCH.											
Period 1:											
Alfalfa hay	6,629.3	646.0	5,983.3	752.0	216.8	2,034.4	2,858.4	121.8	166.5	2,983.2	28,740.3
Starch	2,729.1	6.7	2,722.4	9.5	.....	3.4	2,709.5	.....	1.6	1,191.0	11,202.5
Total fed	9,358.4	652.7	8,705.7	761.5	216.8	2,037.7	5,567.9	121.8	168.1	4,174.2	39,942.8
Refused	537.4	40.4	497.0	42.3	12.9	223.1	211.9	6.8	9.5	241.6	2,303.7
Amount eaten	8,821.0	612.3	8,208.7	719.2	203.9	1,814.6	5,356.0	115.0	158.6	3,932.6	37,639.1
Feces	2,746.8	339.6	2,407.2	287.4	25.2	1,102.2	904.4	87.9	58.7	1,272.7	12,307.6
Amount digested	6,074.2	272.7	5,801.5	431.8	178.7	712.4	4,451.6	27.1	99.9	2,659.9	25,331.5
Percentage digestibility	68.86	44.54	70.68	60.04	87.64	39.26	83.11	23.57	62.99	67.64	67.30
Period 3:											
Alfalfa hay	3,747.7	330.8	3,416.9	410.6	120.3	1,194.1	1,625.8	66.1	91.3	1,684.3	16,452.03
Starch	1,551.1	3.8	1,547.3	5.4	.....	1.9	1,540.0	.....	.9	676.9	6,367.03
Total eaten	5,298.8	334.6	4,964.2	416.0	120.3	1,196.0	3,165.8	66.1	92.2	2,361.2	22,819.06
Feces	1,710.0	171.1	1,538.9	182.5	13.4	695.5	595.3	52.1	34.3	801.9	7,763.81
Amount digested	3,588.8	163.5	3,425.3	233.5	106.9	500.5	2,570.5	14.0	57.9	1,559.3	15,055.25
Percentage digestibility	67.73	48.86	69.00	56.13	88.86	41.85	81.20	21.18	62.80	66.04	65.98
Perod 2:											
Alfalfa hay	2,645.4	233.5	2,411.9	289.8	84.9	842.9	1,148.3	46.6	64.44	1,188.9	11,613.04
Starch	1,105.0	2.7	1,102.3	3.8	.....	1.4	1,097.1	.....	.6	482.2	4,535.86
Total eaten	3,750.4	236.2	3,514.2	293.6	84.9	844.3	2,245.4	46.6	65.0	1,671.1	16,148.90
Feces	1,120.6	124.2	996.4	111.8	9.7	464.7	372.0	38.3	21.9	522.4	5,075.81
Amount digested	2,629.8	112.0	2,517.8	181.8	75.2	379.6	1,873.4	8.3	43.1	1,148.7	11,073.09
Percentage digestibility	70.12	47.42	71.65	61.92	88.57	44.96	83.43	17.81	66.31	68.74	68.57
Period 4:											
Alfalfa hay	1,754.0	162.7	1,591.3	199.3	53.1	516.8	791.7	30.5	43.2	788.9	7,599.76
Starch	726.0	1.8	724.2	2.5	.....	.9	720.8	.....	.4	316.8	2,980.12
Total eaten	2,480.0	164.5	2,315.5	201.8	53.1	517.7	1,512.5	30.5	43.6	1,105.7	10,579.88
Feces	728.9	84.3	644.4	74.4	6.0	296.1	240.7	27.1	15.4	343.3	3,334.40
Amount digested	1,751.1	80.0	1,671.1	127.4	47.1	221.6	1,271.8	3.4	28.2	762.4	7,245.48
Percentage digestibility	70.61	48.63	72.17	63.13	88.70	42.80	84.09	11.15	64.68	68.9	68.48

TABLE 2.—*Urinary excretion*

Feed and period.	Weight.	Average specific gravity.	Total solids.		Total nitrogen.		Total carbon.		Energy.	
									Per gram.	Total.
ALFALFA HAY.										
Period 5:	*Gms.*		*Per cent.*	*Gms.*	*Per cent.*	*Gms.*	*Per cent.*	*Gms.*	*Calories.*	*Calories.*
Collected	95,884	1.041	8.26	7,920.0	1.347	1,291.6	2.040	1,956.0	.1990	19,080.9
Spilled	1,245	.....	.....	33.1	.....	5.4	.....	8.2	.....	79.8
Total, 10 days	97,129	.....	.....	7,953.1	.....	1,297.0	.....	1,964.2	.....	19,160.7
Average	9,712.9	.....	.....	795.3	.....	129.7	.....	196.4	.....	1,916.1
Period 6:										
Collected	90,763	1.035	6.08	5,518.4	1.097	995.7	1.647	1,494.9	.1619	14,694.5
Spilled	1,451	.....	.....	75.4	.....	13.6	.....	20.4	.....	200.7
Total, 10 days	92,214	.....	.....	5,593.8	.....	1,009.3	.....	1,515.3	.....	14,895.2
Average	9,221.4	.....	.....	559.4	.....	100.9	.....	151.5	.....	1,489.5
Period 7:										
Collected	66,059	1.068	6.01	3,970.1	1.068	705.5	1.631	1,077.4	.1510	9,974.9
Average	6,605.9	.....	.....	397.0	.....	70.6	.....	107.7	.....	997.5
ALFALFA HAY AND STARCH.										
Period 1:										
Collected	68,544	1.044	8.768	6,009.9	.948	649.8	1.995	1,367.5	.1745	11,960.9
Spilled	2,943	.....	.....	157.2	.....	17.9	.....	37.7	.....	329.5
Total, 10 days	71,487	.....	.....	6,167.1	.....	667.7	.....	1,405.2	.....	12,290.4
Average	7,148.7	.....	.....	616.7	.....	66.7	.....	140.5	.....	1,229.0
Period 3:										
Collected	71,192	1.0302	4.45	3,168.0	.696	495.5	1.406	1,001.0	.1263	8,991.5
Spilled	805	.....	.....	12.1	.238	1.9	.....	3.8	.....	34.5
Total, 10 days	71,997	.....	.....	3,180.1	.....	497.4	.....	1,004.8	.....	9,026.0
Average	7,199.7	.....	.....	318.0	.....	49.7	.....	100.5	.....	902.6
Period 2:										
Collected, 10 days	60,057	1.030	5.586	3,354.8	.718	431.2	1.304	783.1	.1221	7,333.0
Average	6,005.7	.....	.....	.....	.....	43.1	.....	78.3	.....	733.3
Period 4:										
Collected, 10 days	39,042	1.0308	5.533	2,160.2	.899	351.0	1.506	588.0	.1447	5,649.4
Average	3,904.2	.....	.....	216.0	.....	35.1	.....	58.8	.....	564.9

TABLE 3.—*Average daily production of epidermal tissue*

Factor.	In growth of hair.	In brushings.	Total.
Dry matter.....gm..	2.20	9.19	11.39
Nitrogen.....gm..	0.33	.73	1.06
Carbon.....gm..	1.09	3.96	5.05
Energy.....Cal..	11.99	43.13	55.12

TABLE 4.—*Gaseous excretion*

Feed and period.	Water.	Carbon dioxid.	In combustible gases.		Methane computed from carbon.	Ratio, hydrogen to carbon.
			Hydrogen.	Carbon.		
ALFALFA HAY.						
Period 5:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
First day	7,101.04	5,570.91	43.35	132.19	176.65	3.049
Second day	6,889.56	5,439.04	41.44	127.01	169.72	3.065
Average	6,995.30	5,504.98	42.40	129.60	173.19	3.057
Period 6:						
First day	5,570.20	4,597.76	34.00	102.50	136.96	3.015
Second day	5,341.17	4,539.40	32.89	96.17	128.51	2.924
Average	5,458.69	4,568.58	33.45	99.34	132.74	2.970
Period 7:						
First day	3,525.93	3,266.36	29.39	60.30	80.57	2.052
Second day	3,394.18	3,238.68	22.89	58.22	77.80	2.543
Average	3,460.06	3,252.52	26.14	59.26	79.19	2.267
ALFALFA HAY AND STARCH.						
Period 1:						
First day	10,423.97	7,068.02	56.18	172.75	230.85	3.075
Second day	9,632.29	7,022.94	53.61	164.20	219.43	3.063
Average	10,028.13	7,045.48	54.90	168.48	225.14	3.069
Period 3:						
First day	5,257.87	4,732.27	37.28	115.62	154.50	3.101
Second day	5,247.31	4,717.45	38.19	117.76	157.36	3.084
Average	5,252.59	4,724.86	37.74	116.69	155.93	3.092
Period 2:						
First day	4,559.94	3,929.83	26.86	82.98	110.88	3.089
Second day	4,208.61	3,889.07	27.36	84.65	113.11	3.094
Average	4,384.28	3,903.45	27.11	83.82	112.00	3.092
Period 4:						
First day	3,175.56	3,158.00	20.03	63.16	84.39	3.153
Second day	3,161.98	3,109.36	19.62	61.57	82.28	3.138
Average	3,168.77	3,133.68	19.83	62.37	83.34	3.145

TABLE 5.—*Heat emission*

Feed and period.	By radiation and conduction.	As latent heat of water vapor.	Total.
Period 5: ALFALFA HAY.	*Calories.*	*Calories.*	*Calories.*
First day	8,812.59	4,100.69	12,913.28
Second day	8,651.67	4,039.94	12,691.61
Average	8,732.13	4,070.32	12,802.45
Period 6:			
First day	7,742.57	3,273.23	11,015.80
Second day	7,661.46	3,152.17	10,813.63
Average	7,702.02	3,212.70	10,914.72
Period 7:			
First day	6,120.28	2,082.40	8,202.68
Second day	6,271.53	1,996.61	8,268.14
Average	6,195.91	2,039.51	8,235.41

TABLE 5—*Heat emission*—Continued

Feed and period.	By radiation and conduction.	As latent heat of water vapor.	Total.
ALFALFA HAY AND STARCH.			
Period 1:	*Calories.*	*Calories.*	*Calories.*
First day	10,731.85	4,860.76	15,592.61
Second day	10,619.21	4,887.53	15,506.74
Average	10,675.53	4,874.15	15,549.67
Period 3:			
First day	7,744.58	3,086.37	10,830.95
Second day	7,770.84	3,071.71	10,842.55
Average	7,757.71	3,079.04	10,836.75
Period 2:			
First day	7,075.60	2,583.70	9,659.30
Second day	6,986.47	2,457.77	9,444.24
Average	7,031.04	2,520.73	9,551.77
Period 4:			
First day	6,108.95	1,880.96	7,989.91
Second day	6,102.51	1,862.38	7,964.89
Average	6,105.73	1,871.67	7,977.4

TABLE 6.—*Corrections of heat emission for gains by body*[a]

Feed and period.	Gain—			Mean body temperature.	Calorimeter temperature.	Correction.
	Protein.[b]	Fat.	Water.			
ALFALFA HAY.						
Period 5:	*Gm.*	*Gm.*	*Gm.*	°C.	°C.	*Cal.*
First day	+ 61.2	+178.4	−8,558	.....	.....	−176
Second day	+ 61.2	+232.3	+2,513	.....	.....	+ 56
Average	+ 61.2	+205.3	−3,022	38.58	17.66	− 60
Period 6:						
First day	+ 57.0	+ 46.0	−9,393	.....	.....	−197
Second day	+ 57.0	+ 75.8	+1,242	.....	.....	+ 28
Average	+ 57.0	+ 60.9	−4,076	38.70	17.67	− 85
Period 7:						
First day	− 43.2	−160.2	− 604	.....	.....	− 15
Second day	− 43.2	−147.7	− 52	.....	.....	− 3
Average	− 43.2	−154.0	− 328	38.00	17.71	− 9
ALFALFA HAY AND STARCH.						
Period 1:						
First day	+192.0	+409.2	−7,403	.....	.....	−148
Second day	+192.0	+436.5	+5,938	.....	.....	+131
Average	+192.0	+422.9	− 733	38.67	17.76	− 8
Period 3:						
First day	+ 42.6	+ 32.9	−3,634	.....	.....	− 75
Second day	+ 42.6	+ 35.3	+1,117	.....	.....	+ 25
Average	+ 42.6	+ 34.1	−1,259	38.50	17.69	− 25
Period 2:						
First day	− 6.6	−112.3	−1,480	.....	.....	− 32
Second day	− 6.6	−100.0	− 369	.....	.....	− 9
Average	− 6.6	−106.2	− 924	38.28	17.72	− 21
Period 4:						
First day	− 48.0	−262.3	+ 102	.....	.....	− 2
Second day	− 48.0	−242.8	−1,099	.....	.....	− 26
Average	− 48.0	−252.6	− 499	38.14	17.72	− 14

[a] Estimated specific heats; protein 0.30, fat 0.66.

[b] Average of entire digestion period.

TABLE 7.—*Corrections of heat emission for changes of body temperature in 48 hours*

Feed and period.	Live weight when leaving calorimeter.	Body temperature.[a]			Corrections for body temperature.[b]
		Entering.	Leaving.	Difference.	
ALFALFA HAY.	*Kgm.*	*°C.*	*°C.*	*°C.*	*Cal.*
Period 5	423.2	38.33	38.83	+0.50	+169
Period 6	412.2	38.67	38.72	+0.05	+ 16
Period 7	383.0	37.44	38.56	+1.12	+343
ALFALFA HAY AND STARCH.					
Period 1	409.8	38.67	38.67	0.00	0
Period 3	374.4	38.33	38.67	+0.34	+108
Period 2	398.0	38.22	38.33	+0.11	+ 33
Period 4	364.7	37.94	38.33	+0.39	+114

[a] Taken in Fahrenheit degrees and reduced. [b] Specific heat of body, 0.8.

TABLE 8.—*Daily heat production*

Feed and period.	Heat emission.	Correction for body temperature[a] (Table 7).	Correction for body gain per day (Table 6).	Observed heat production.	Computed heat production.	Computed ÷ observed.
ALFALFA HAY.						
Period 5:	*Calories.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*	*Per cent.*
First day	12,913.3	+ 85	−176	12,822.3	13,030.7	101.6
Second day	12,691.6	+ 85	+ 56	12,832.6	12,611.1	98.3
Average	12,802.5	+ 85	− 60	12,827.5	12,820.9	99.9
Period 6:						
First day	11,015.8	+ 8	−197	10,826.8	11,144.1	102.9
Second day	10,813.6	+ 8	+ 28	10,849.6	10,981.4	101.2
Average	10,914.7	+ 8	− 85	10,837.7	11,062.8	102.0
Period 7:						
First day	8,202.7	+172	− 15	8,359.7	8,542.4	102.2
Second day	8,268.1	+172	− 3	8,437.1	8,460.6	100.3
Average	8,235.4	+172	− 9	8,398.4	8,501.5	101.2
ALFALFA HAY AND STARCH.						
Period 1:						
First day	15,592.6	0	−148	15,444.6	15,985.1	103.5
Second day	15,506.7	0	+131	15,637.7	15,878.1	101.5
Average	15,549.7	0	− 8	15,541.7	15,931.6	102.5
Period 3:						
First day	10,831.0	+ 54	− 75	10,810.0	11,420.8	105.6
Second day	10,842.6	+ 54	+ 25	10,921.6	11,419.6	104.6
Average	10,836.8	+ 54	− 25	10,865.8	11,420.2	105.1
Period 2:						
First day	9,659.3	+ 16	− 32	9,643.3	9,909.6	102.8
Second day	9,444.2	+ 16	− 9	9,451.2	9,763.0	103.3
Average	9,551.8	+ 16	− 21	9,546.8	9,836.3	103.0
Period 4:						
First day	7,989.9	+ 57	− 2	8,044.9	8,264.9	102.7
Second day	7,964.9	+ 57	− 26	7,995.9	8,107.8	101.4
Average	7,977.4	+ 57	− 14	8,020.4	8,186.4	102.0

[a] Assuming the correction for 48 hours (Table 7) to be equally divided between the two days.

SOIL FACTORS AFFECTING THE TOXICITY OF ALKALI

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INTRODUCTION

In alkali studies carried out at the Utah Experiment Station during a number of years, variations in toxicity under different conditions have been very evident. This has already been reported by one of us,[1] in results wherein the various alkali salts and particularly the carbonates were injurious in lower concentrations in sand than in loam. Other soil factors were also found to influence the action of soluble salts on plants. In order to gain more information regarding these factors, the investigations herein reported were undertaken, since it was realized that no adequate idea of the alkali problem could be had without an understanding of the factors involved.

Millions of acres of land in the arid part of America contain certain soluble salts in sufficient concentration to interfere with the best growth of crops. The value of much of this land is uncertain, since the salt content is near the concentration that renders it worthless. It is important, therefore, to know as nearly as possible just what the critical concentrations are. Hilgard and other early investigators contributed much to our knowledge of alkali lands, but their investigations did not make clear all the factors involved. This work is an attempt to determine with more exactness the quantities of the various salts that prohibit crop growth under different soil conditions.

The earlier paper[1] reviews the important literature on the subject; consequently no literature is reported in the present paper.

METHODS OF EXPERIMENTATION

The first method used was the direct empirical experiment of growing crops in prepared alkali soils in glass tumblers. Over 12,000 tumblers were planted in this experiment. The general method of procedure was exactly the same as was used previously[2] in studying the effect of different salts and combinations of salts. The tumblers were made to a uniform weight by placing washed gravel in the bottom. Two hundred gms. of soil, to which the salts had been added in solution as required in the experiment, were placed in each tumbler, and 10 kernels of New Zealand wheat planted in each. Daily notes were taken on the number of plants up in each tumbler and on any other observable data. The

[1] HARRIS, F. S. EFFECT OF ALKALI SALTS IN SOILS ON THE GERMINATION AND GROWTH OF CROPS. *In* Jour. Agr. Research, v. 5, no. 1, p. 1–53, 48 fig. 1915. Literature cited, p. 52–53.

[2] HARRIS, F. S. OP CIT.

tumblers were made up to weight with distilled water as often as the loss became appreciable. On the twenty-first day after planting, the crop was harvested by cutting the plants at the surface of the ground with small scissors. The height of each plant and the number of leaves were

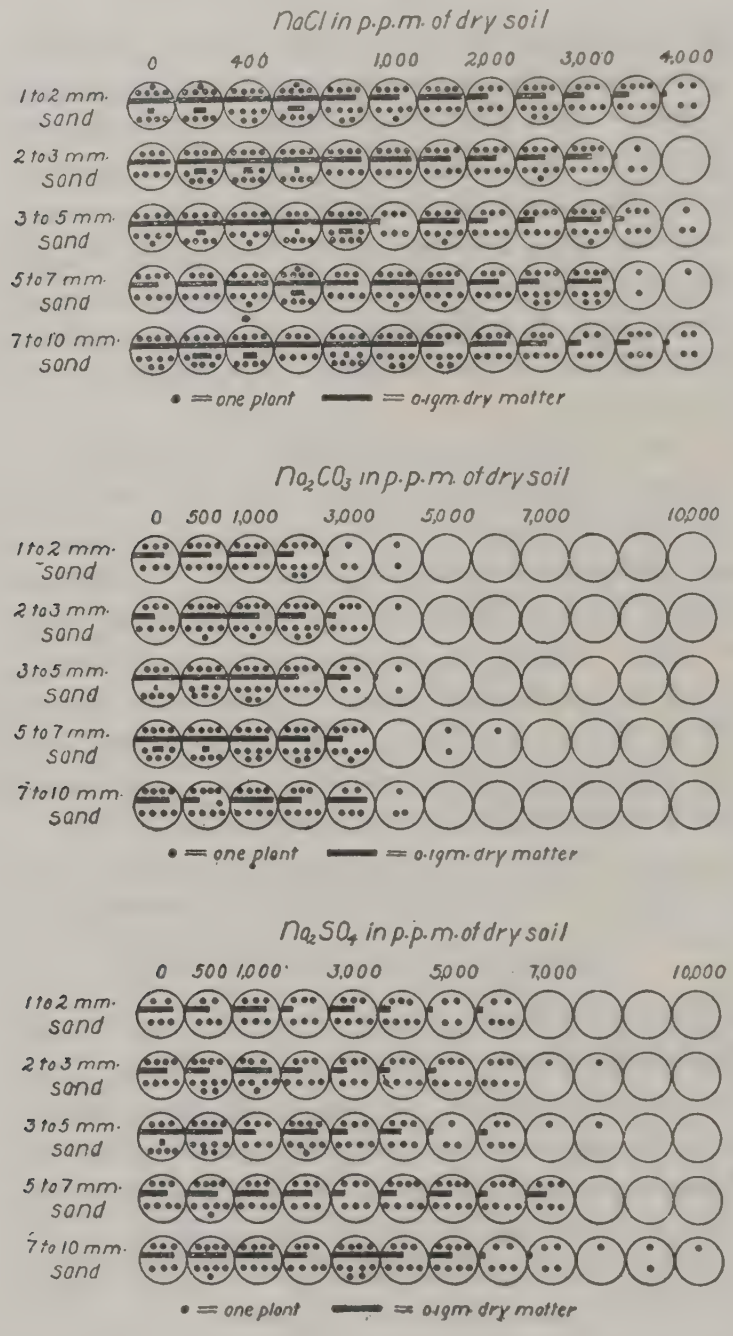

FIG. 1.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in quartz sand of different sizes containing sodium chlorid. Sodium carbonate and sodium sulphate added in various concentrations. Moisture content maintained at 20 per cent throughout.

recorded. The plants from each tumbler were placed in an envelope and dried in an oven, and the dry weight was determined. The 3-week period was arbitrarily chosen, because by that time nearly all the plants that would germinate had done so, and the growth ceased to be vigorous.

It was found that the dry weight at harvest gave the best figures for comparison. The number of plants germinated, the average number of days required to germinate, the average height of plants, and the aver-

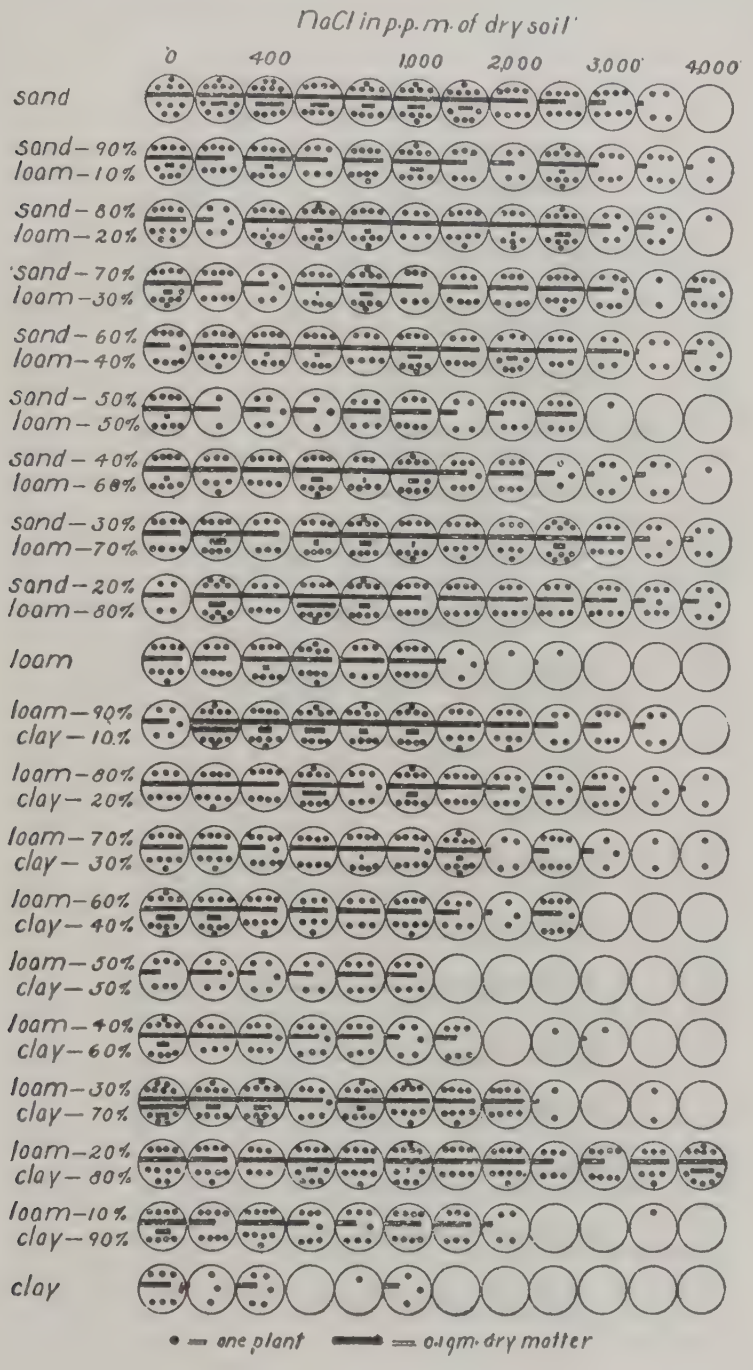

FIG. 2.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils of different texture containing sodium chlorid added in various concentrations. Moisture content maintained at 20 per cent throughout.

age number of leaves were also used as indicators of plants growth. In these experiments only the three most important alkali salts, sodium chlorid, sodium carbonate, and sodium sulphate, were used. The relative toxicity of most of the alkali salts was reported in the earlier paper.

EFFECT OF SIZE OF SOIL PARTICLES

The first factor to be examined was the effect of the size of soil particles on the toxicity of alkali. For this purpose pure quartz sand graded into five sizes was used. The experiment was repeated three times in

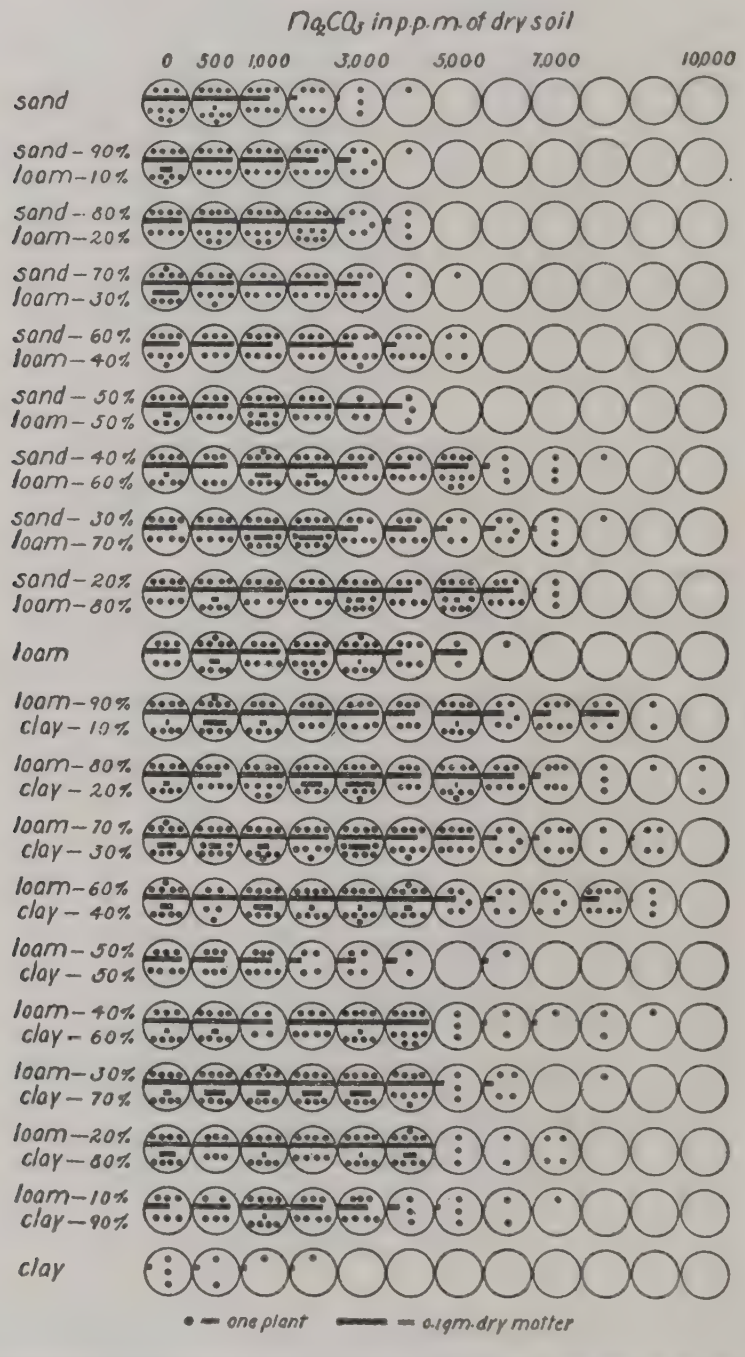

FIG. 3.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils of different texture containing sodium carbonate added in various concentrations. Moisture content maintained at 20 per cent throughout.

two different years, twice at 20 per cent moisture and once at 10 per cent. The details of the make-up of the tumblers and the results are shown in figure 1.

The size of particles alone, as shown by the quartz sand, seemed to have no appreciable effect on the toxicity of the salts. The different moisture content shows a marked difference here as elsewhere. The results with sand are the only ones showing the carbonate more toxic than the chlorid on the basis of the amount of salt added.

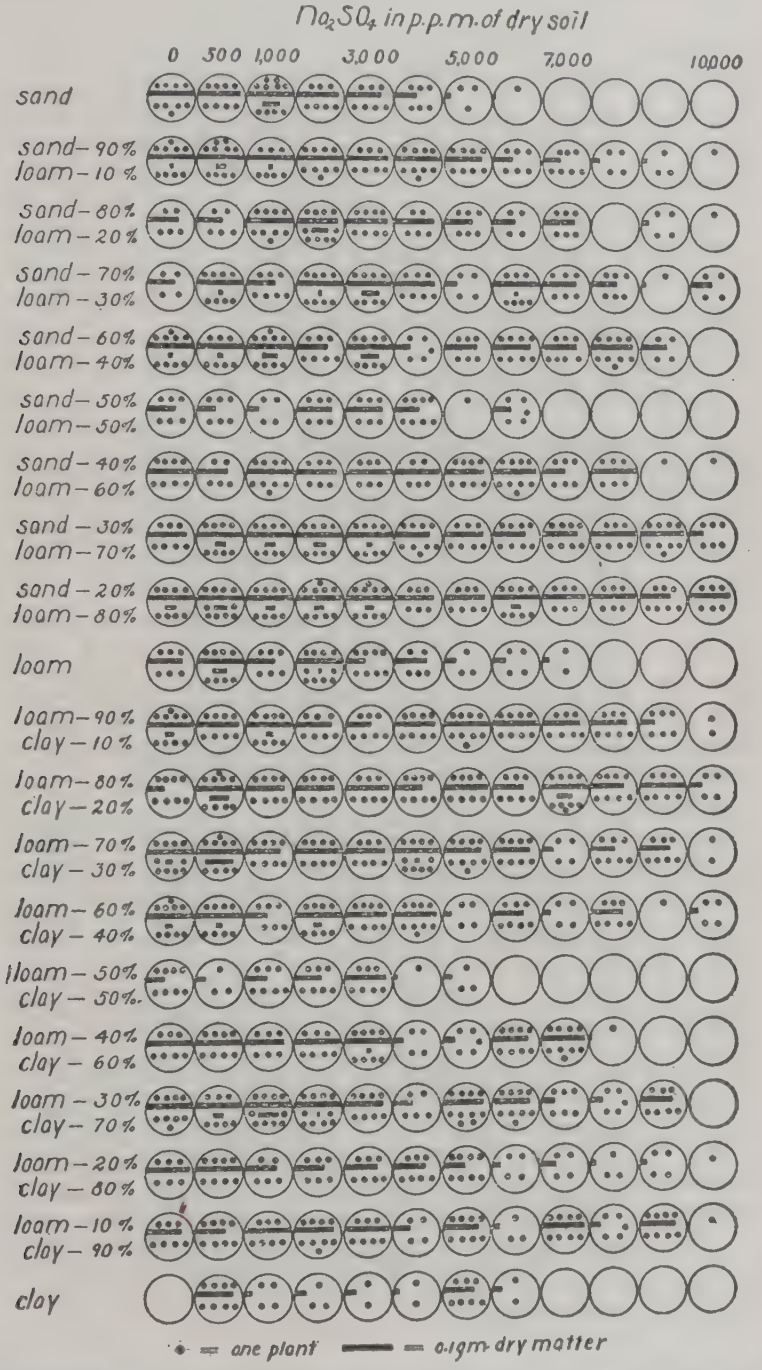

FIG. 4.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils of different texture containing sodium sulphate added in various concentrations. Moisture content maintained at 20 per cent throughout.

As a continuation of the study of the effect of size of particles or texture, various combinations were used, including soils varying from sand through loam to a brickyard clay. The loam contained more organic matter than either of the others. This condition is usually found in

natural soils. A series was conducted with local sand, Greenville loam, and clay, and mixtures of half and half of these. Later, another series was conducted using more grades of mixtures. Owing probably to weather conditions, the second series grew better as a whole than the

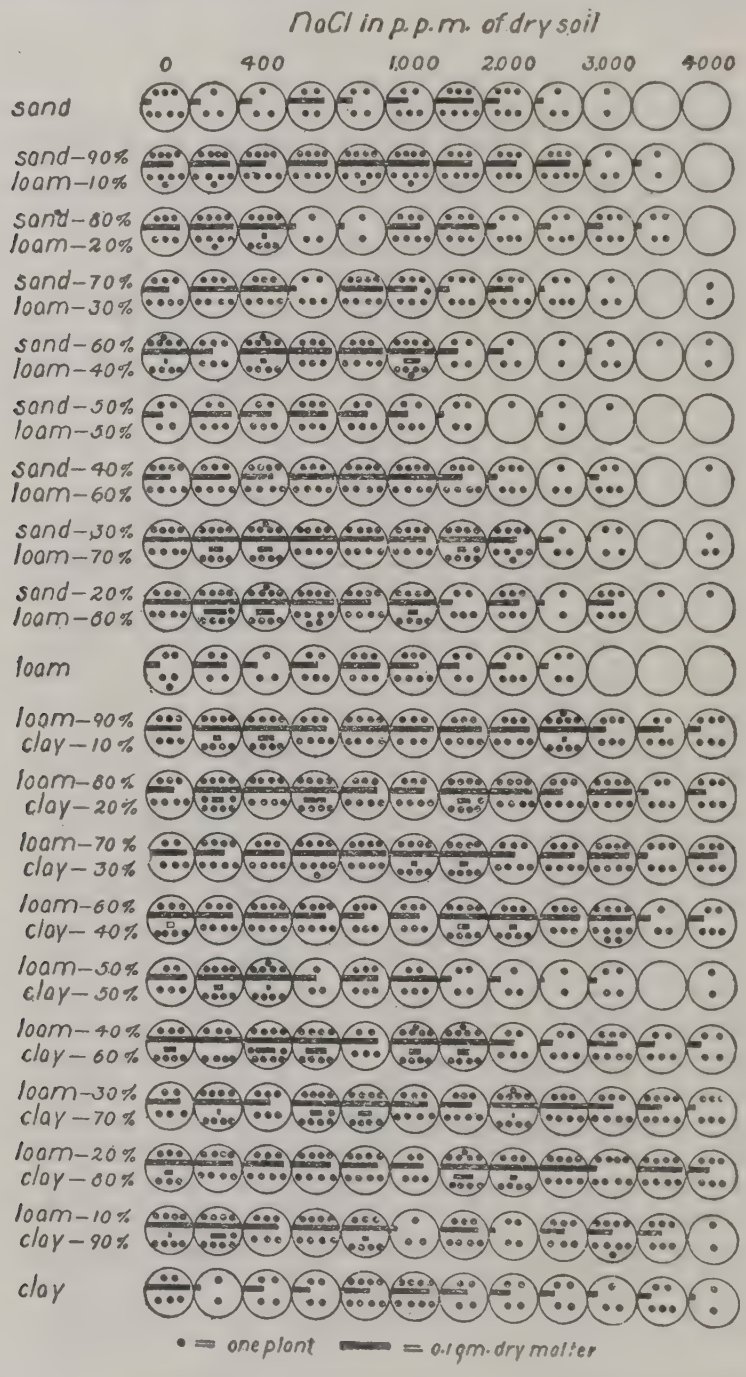

FIG. 5.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils containing sodium chlorid added in various concentrations. Soils maintained at an equivalent moisture content.

first; but, since the figures are equally distributed, they can be placed in one table for study. All soil was maintained at 20 per cent moisture on the basis of the dry soil. The details of the experiment and the results are shown in figures 2, 3, and 4. It is noticeable from the figures that

the loam is more tolerant than either of the extremes and that the lighter loam soils are more tolerant than the heavier. This is especially noticeable with the carbonates.

FIG. 6.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils of different texture containing sodium carbonate added in various concentrations. Soils maintained at an equivalent moisture content.

It was noticed in this experiment that at 20 per cent moisture content the sand was nearly saturated, while the clay was comparatively dry. In order to overcome the effect of the varying moisture relations when soils of all textures were maintained at 20 per cent, experiments were

conducted with the soils as nearly as possible to an equivalent moisture content or the same relative degree of wetness. Since there was no centrifuge available, this equivalent moisture content was determined by

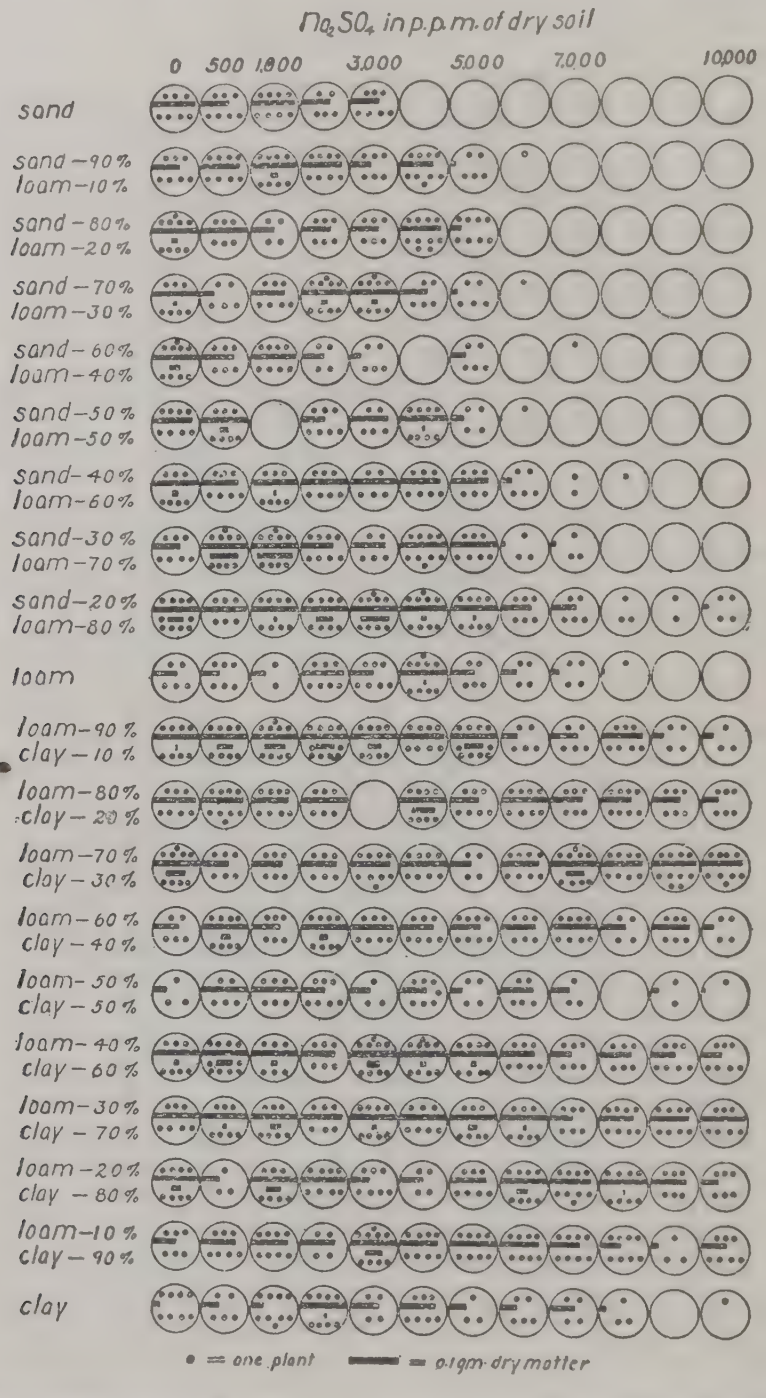

FIG. 7.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in soils of different texture containing sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

placing the soils at various moisture contents in contact with loam at 20 per cent moisture in sealed vessels and observing by the moisture movements the percentage in each case which was in equilibrium with 20 per cent in the loam.

As determined by several duplicates of this method, 10 per cent moisture in the sand, 27 per cent in the clay, and 50 per cent in peat,

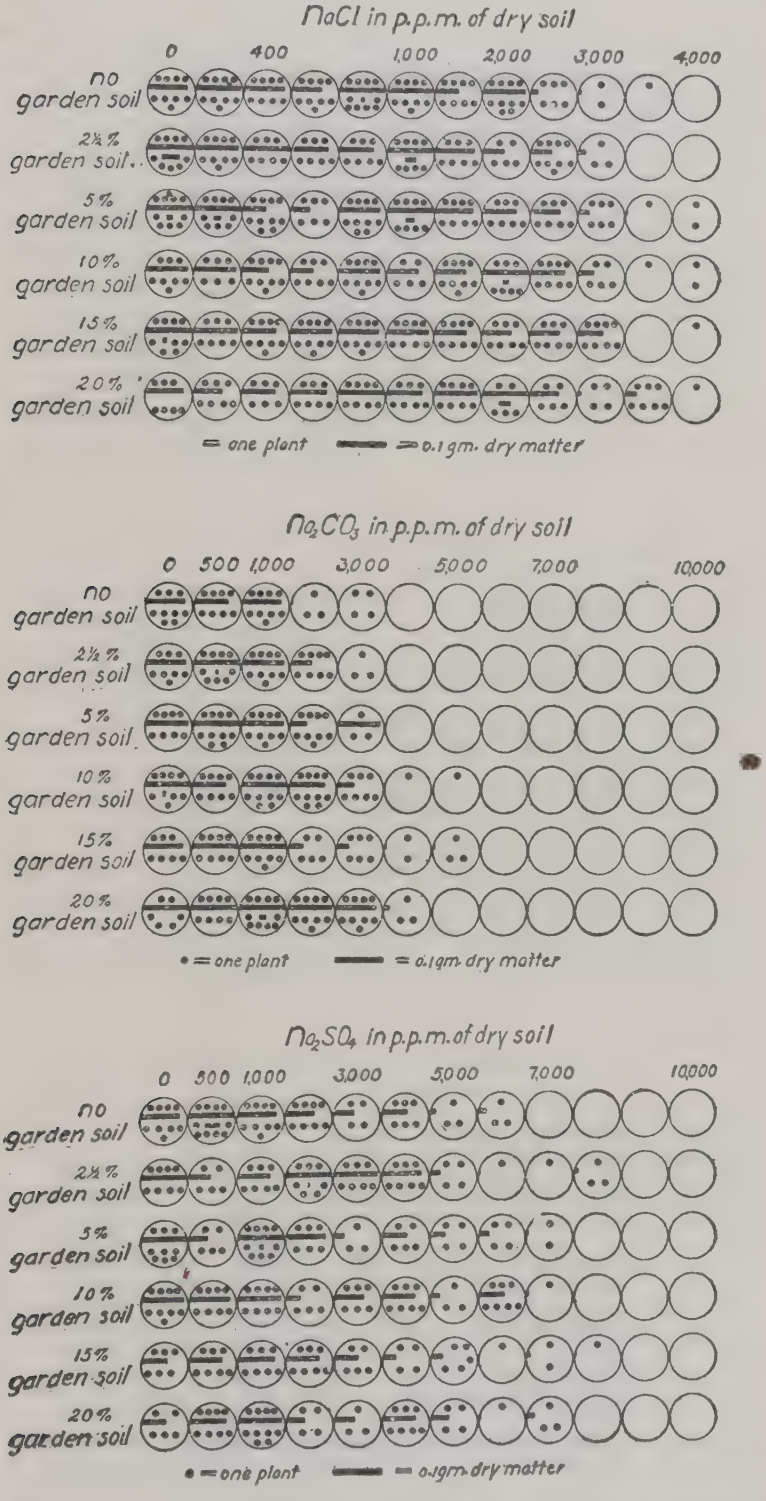

FIG. 8.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand with different amounts of garden soil containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Moisture content maintained at 20 per cent throughout.

used in later experiments, was found to be equivalent to 20 per cent in the loam. As worked out later on the moisture-equivalent centrifuge,

the equivalents were sand 2.66 per cent, loam 23.92 per cent, clay 27.07 per cent, and peat 51.80 per cent; but the first results are probably as

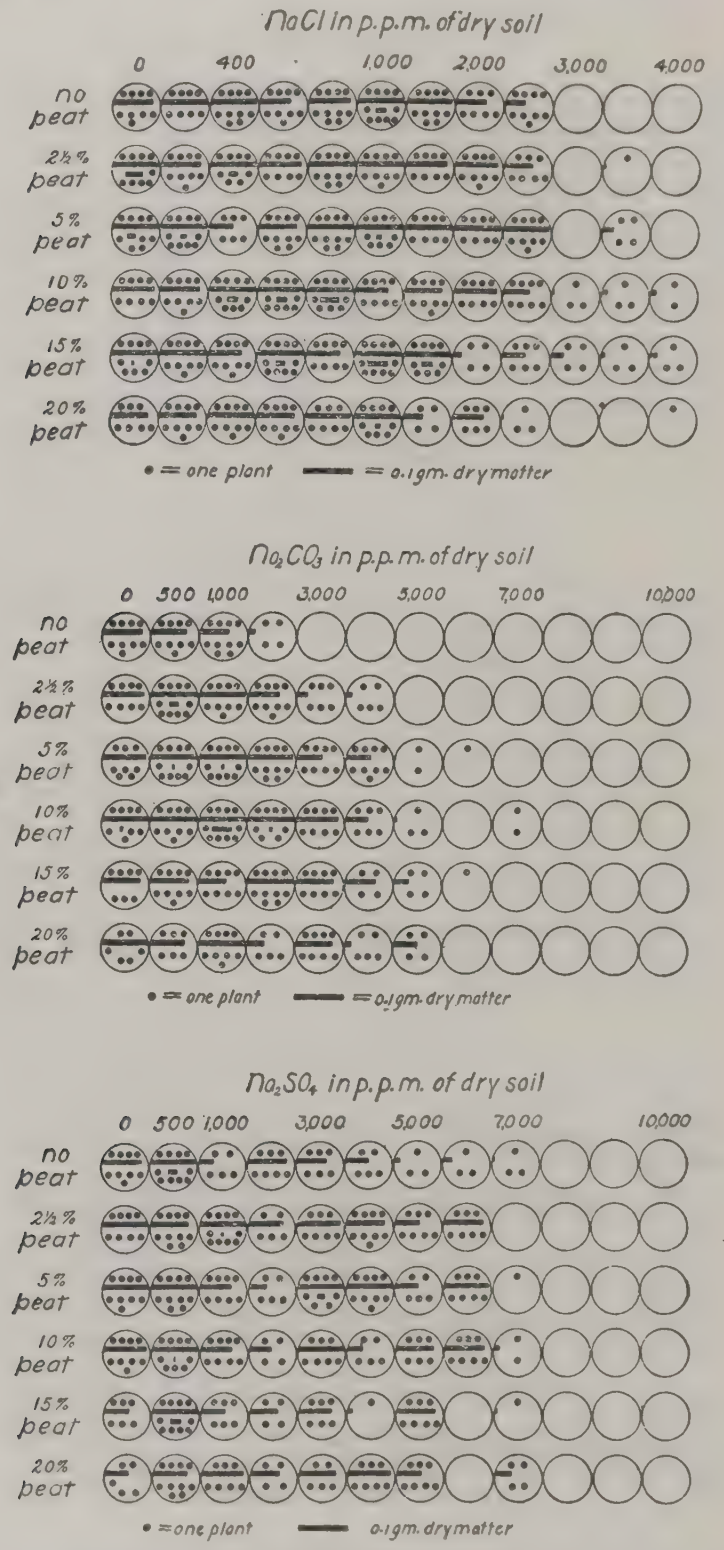

FIG. 9.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand with different amounts of peat containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Moisture content maintained at 20 per cent throughout.

satisfactory for this experiment, since the sand at the lower moisture content would be unworkable. The results of this experiment are

shown in figures 5, 6, and 7. These graphs show but little difference in the tolerance of sand and clay, but the presence of loam greatly increases tolerance for alkali.

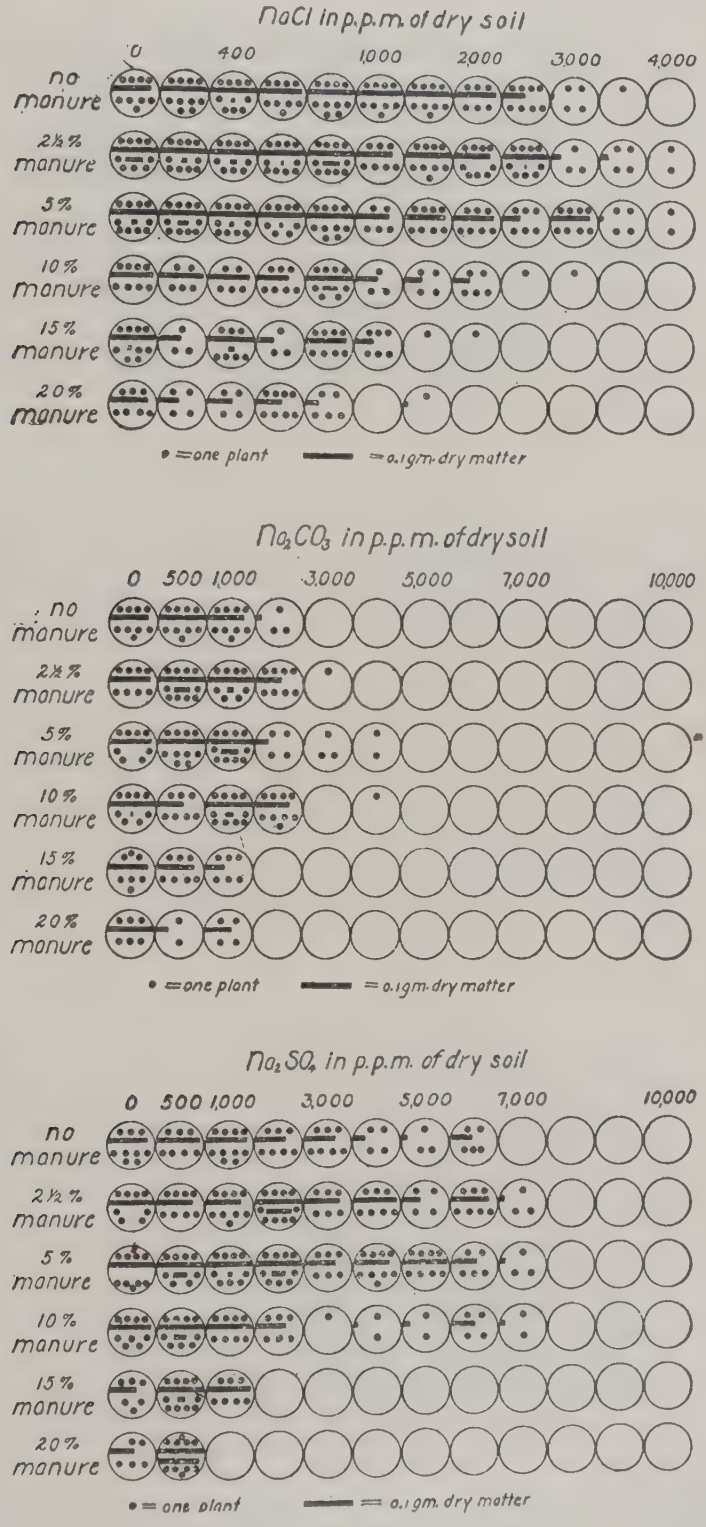

FIG. 10.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand with different amounts of manure containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Moisture content maintained at 20 per cent throughout.

In order to eliminate the effect of the organic matter which was present in the loam and not in the other soils, two series were conducted,

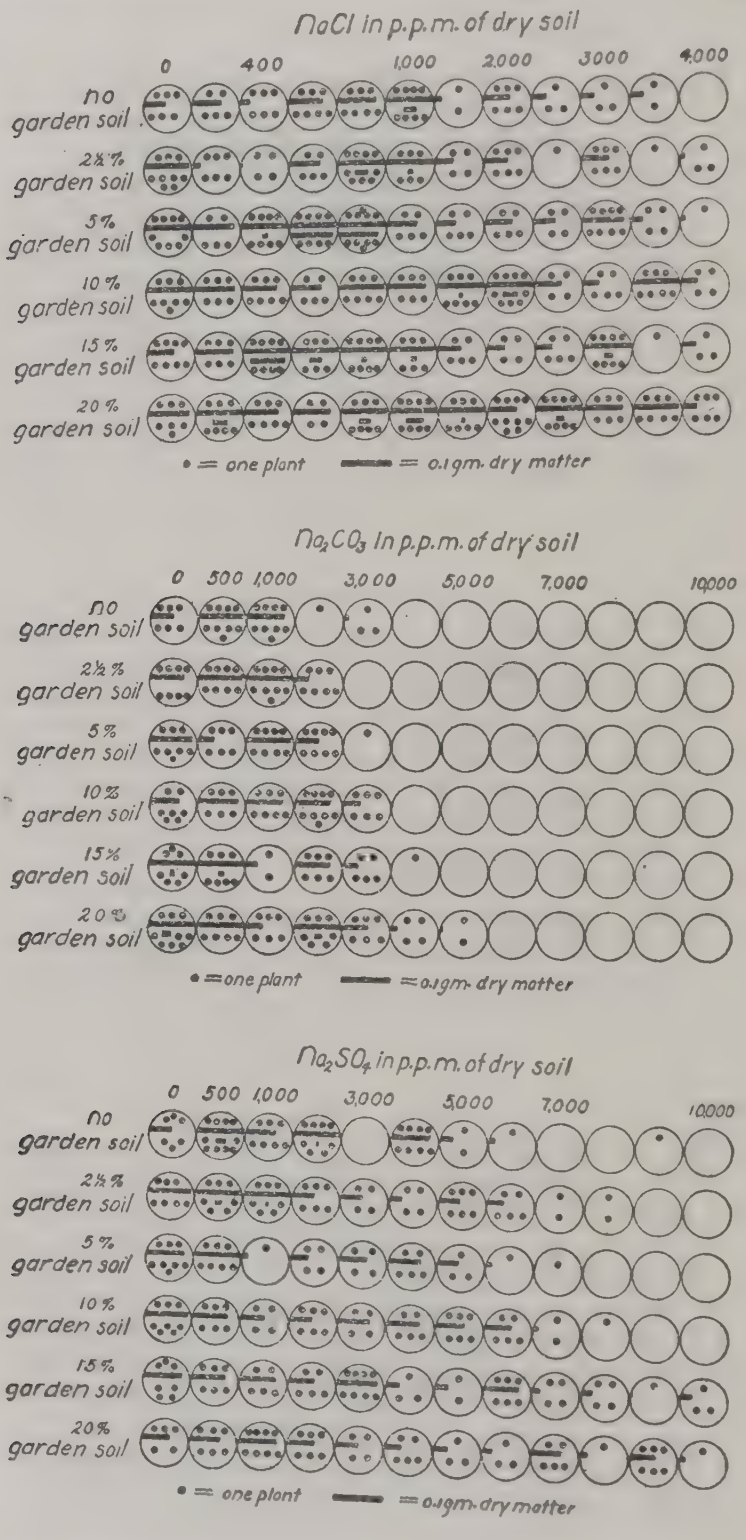

FIG. 11.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand with different amounts of garden soil containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

using only mixtures of clay and sand at an equivalent moisture content. In one series no organic matter was added, and in the other 10 per cent

of sifted peat was added to each. In this case only four concentrations of the salt were used and there were four duplicates of each treatment.

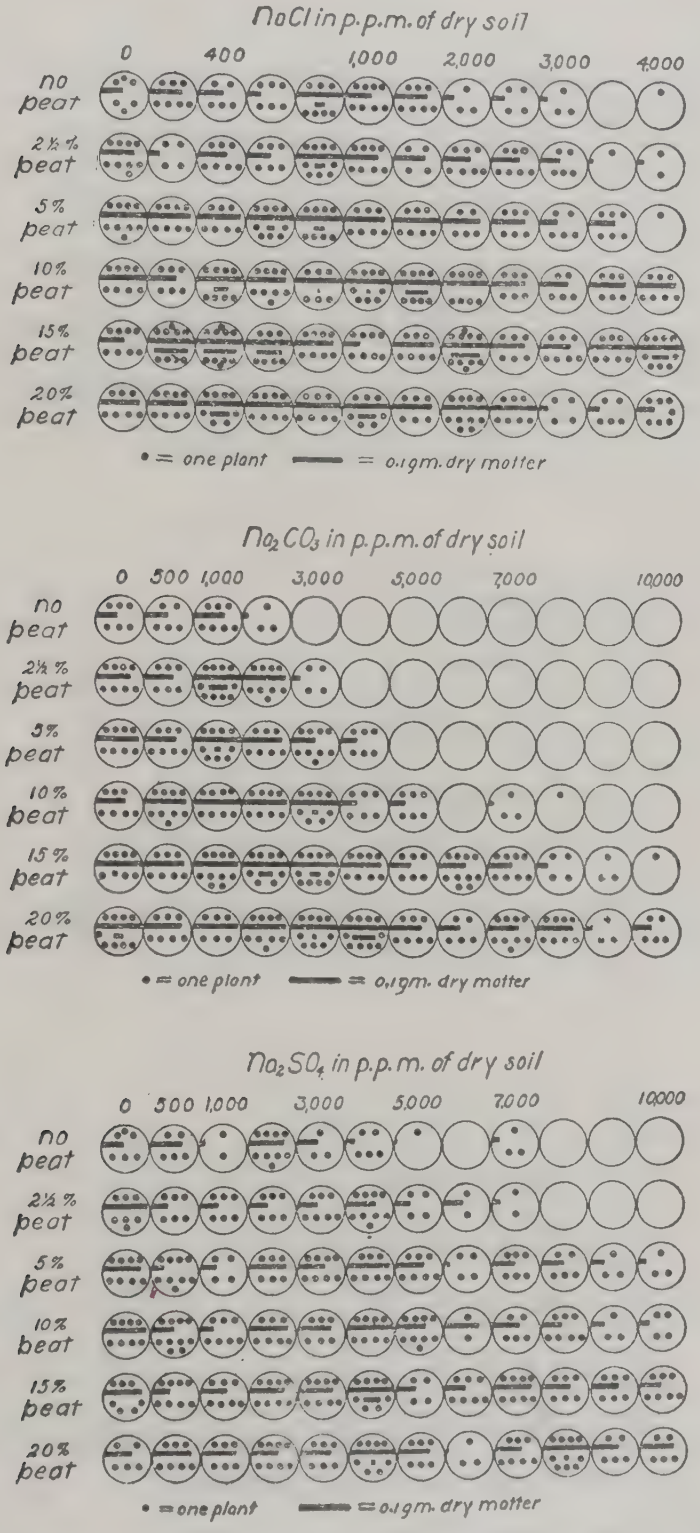

FIG. 12.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand with different amounts of peat containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

These were averaged. The results are given in Tables I and II. These tables show that without the organic matter the heavier soils are more

tolerant at the equivalent moisture content; but with the organic matter added, which greatly increased the equivalent moisture, there is no

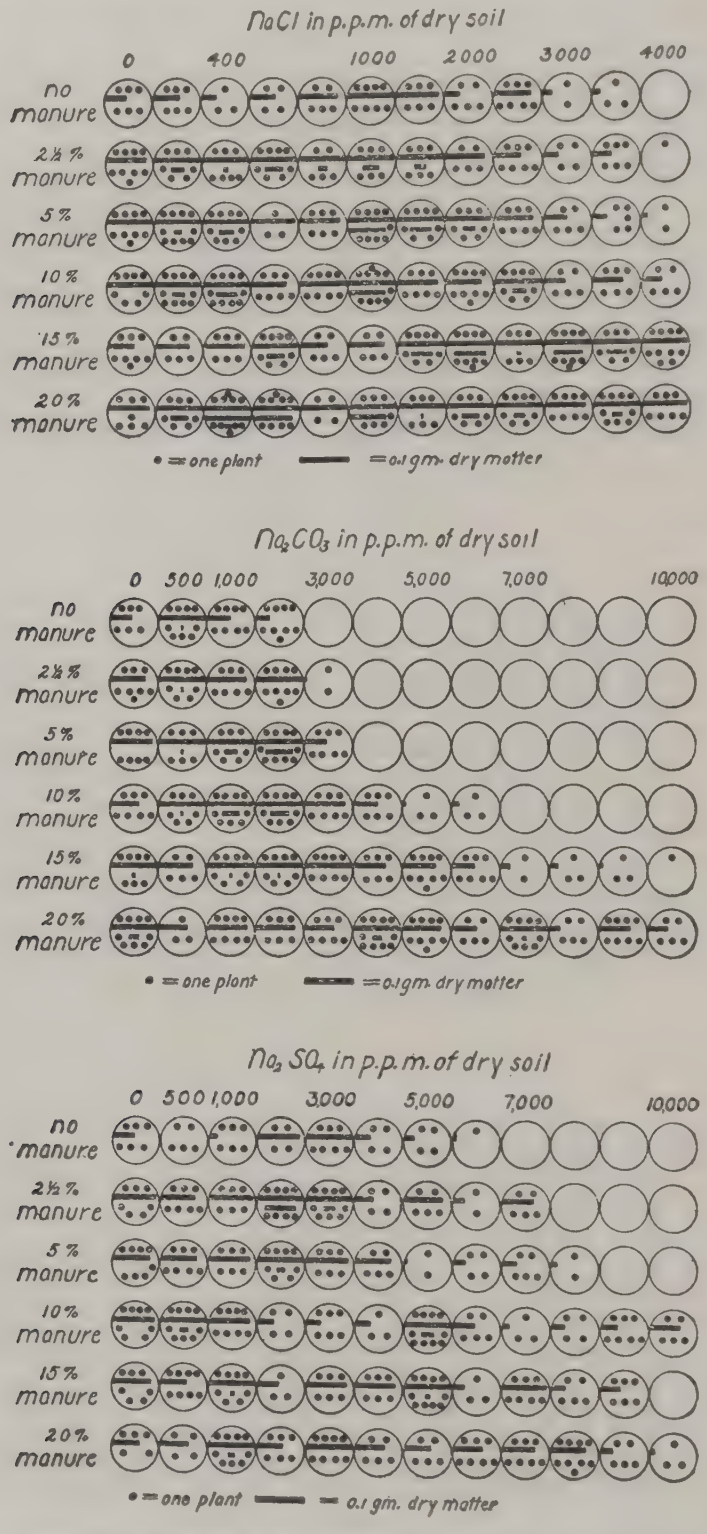

FIG. 13.—Diagram showing the number of wheat plants up and the dry matter produced in sand with different amounts of manure containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils manintained at an equivalent moisture content.

appreciable effect. All these results considered together seem to indicate that loams are more tolerant than sands or clays, and that at the same

moisture content the lighter loams are more tolerant; but if enough more water is added to make them equally wet the heavier soils are more tolerant.

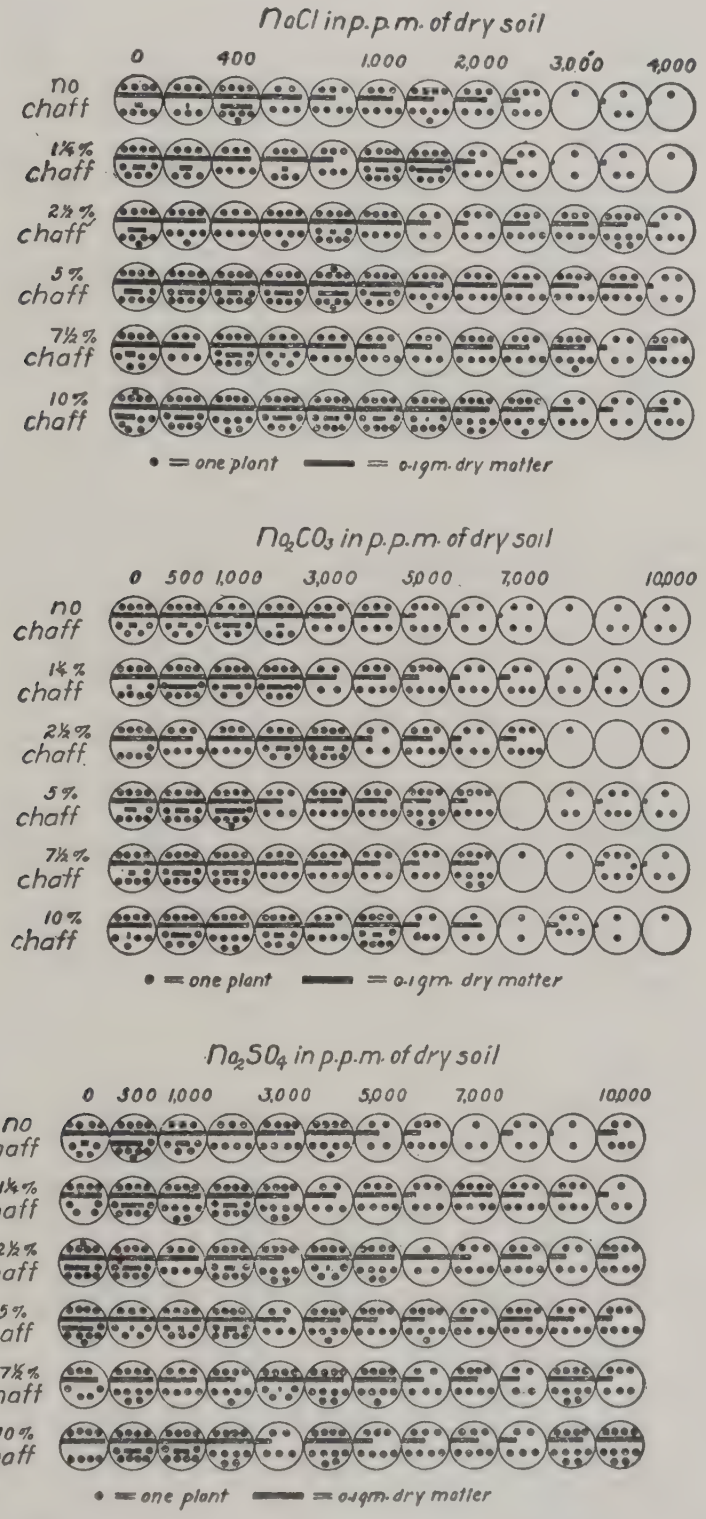

FIG. 14.—Diagram showing the number of wheat plants up and the dry matter produced in loam with different amounts of chaff, containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

TABLE I.—*Average dry matter of wheat (in 0.0001 gm.) produced in 21 days in sand and clay mixtures, with no organic matter and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations. Soils maintained at an equivalent moisture content*

Salt and concentration.	Sand.	Sand 90, clay 10.	Sand 80, clay 20.	Sand 70, clay 30.	Sand 60, clay 40.	Sand 50, clay 50.	Sand 40, clay 60.	Sand 30, clay 70.	Sand 20, clay 80.	Sand 10, clay 90.	Clay.	Average.
P. p. m.												
Control	1,195	1,321	1,156	1,244	1,086	1,188	1,174	1,063	1,190	788	740	1,104
SODIUM CHLORID.												
1,000	1,201	643	669	578	858	917	1,264	775	687	778	1,325	881
2,000	38	7	58	161	608	367	786	318	427	484	918	379
3,000	.....	.....	.....	.....	.....	55	43	127	323	367	500	129
4,000	.....	.....	.....	.....	.....	.....	1	25	88	7	479	55
Average	310	162	182	185	367	335	524	311	381	409	805	361
SODIUM CARBONATE.												
2,000	215	5	89	97	142	439	644	972	764	906	1,217	499
4,000	.....	.....	.....	.....	8	134	18	241	581	436	542	178
6,000	.....	.....	.....	.....	.....	9	49	11	41	216	472	72
8,000	.....	.....	.....	.....	.....	.....	.....	57	9	46	142	23
Average	54	1	22	24	38	161	178	320	349	401	593	194
SODIUM SULPHATE.												
3,000	382	259	651	1,009	518	1,189	199	976	1,082	1,118	1,159	787
6,000	.....	.....	.....	5	.....	88	10	182	199	178	558	111
9,000	.....	.....	.....	.....	.....	.....	.....	.....	1	18	18	4
12,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
Average	95	65	190	255	130	319	52	290	321	329	434	225

TABLE II.—*Average dry matter of wheat (in 0.0001 gm.) produced in 21 days in sand and clay mixtures with 10 per cent peat and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations. Soils maintained at an equivalent moisture content*

Salt and concentration.	Sand.	Sand 90, clay 10.	Sand 80, clay 20.	Sand 70, clay 30.	Sand 60, clay 40.	Sand 50, clay 50.	Sand 40, clay 60.	Sand 30, clay 70.	Sand 20, clay 80.	Sand 10, clay 90.	Clay.	Average.
P. p. m.												
Control	1,073	1,084	943	839	778	1,040	566	653	661	535	864	821
SODIUM CHLORID.												
1,000	1,409	1,053	1,202	1,294	1,305	935	1,502	1,162	1,435	1,039	1,089	1,220
2,000	873	898	1,244	1,280	920	945	975	622	1,192	818	764	957
3,000	272	422	732	529	294	603	684	671	589	646	320	524
4,000	159	125	175	145	64	380	401	278	474	204	202	237
Average	678	625	838	812	646	716	890	683	922	677	594	734
SODIUM CARBONATE.												
2,000	1,070	939	1,328	1,079	1,520	1,094	1,042	374	780	.....	511	974
4,000	1,188	533	931	1,141	1,554	888	750	478	796	.....	558	882
6,000	592	515	889	841	898	874	603	700	717	.....	350	698
8,000	149	157	192	455	586	425	460	673	434	.....	268	380
Average	750	536	835	879	1,140	820	714	556	682	.....	422	734
SODIUM SULPHATE.												
3,000	1,313	1,442	1,083	1,407	1,120	1,156	998	1,002	914	1,218	1,382	1,185
6,000	1,178	1,161	1,152	1,023	1,264	1,191	908	1,255	1,117	1,059	1,000	1,119
9,000	1,077	1,088	1,140	1,070	913	966	703	739	656	725	521	823
12,000	883	873	807	803	800	694	596	761	467	758	712	741
Average	1,113	1,141	1,046	1,076	1,024	1,002	801	939	789	940	904	979

EFFECT OF ORGANIC MATTER

Another factor to be studied separately was the effect of the organic matter of the soil on the toxicity of the alkali. The results given above

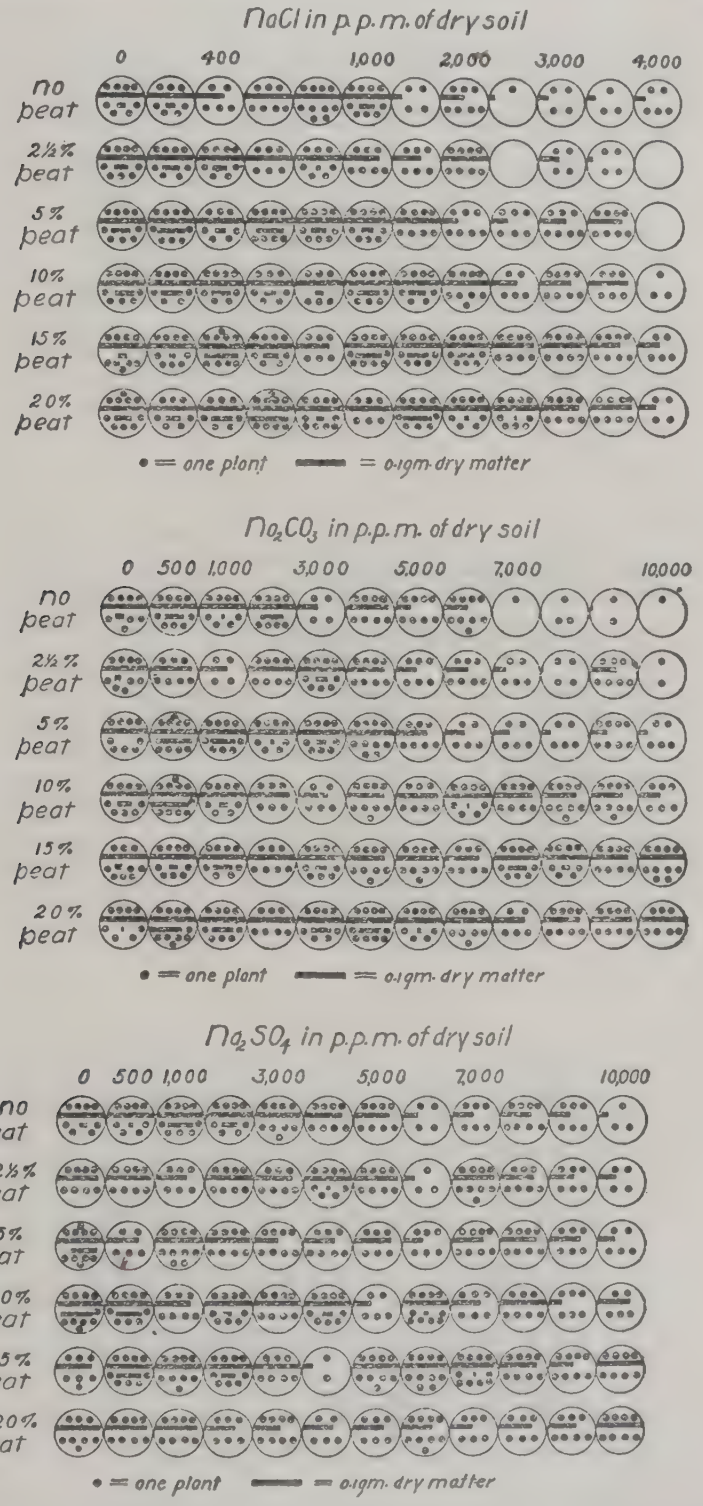

FIG. 15.—Diagram showing the number of heat plants up and the dry matter produced in loam with different amounts of peat containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

seem to indicate quite strikingly that organic matter increases tolerance. Several series of experiments were conducted to show the effect of a rich

garden soil, sifted peat, and sifted manure in varying quantities on the toxicity of alkali in sand at the same and at equivalent moisture con-

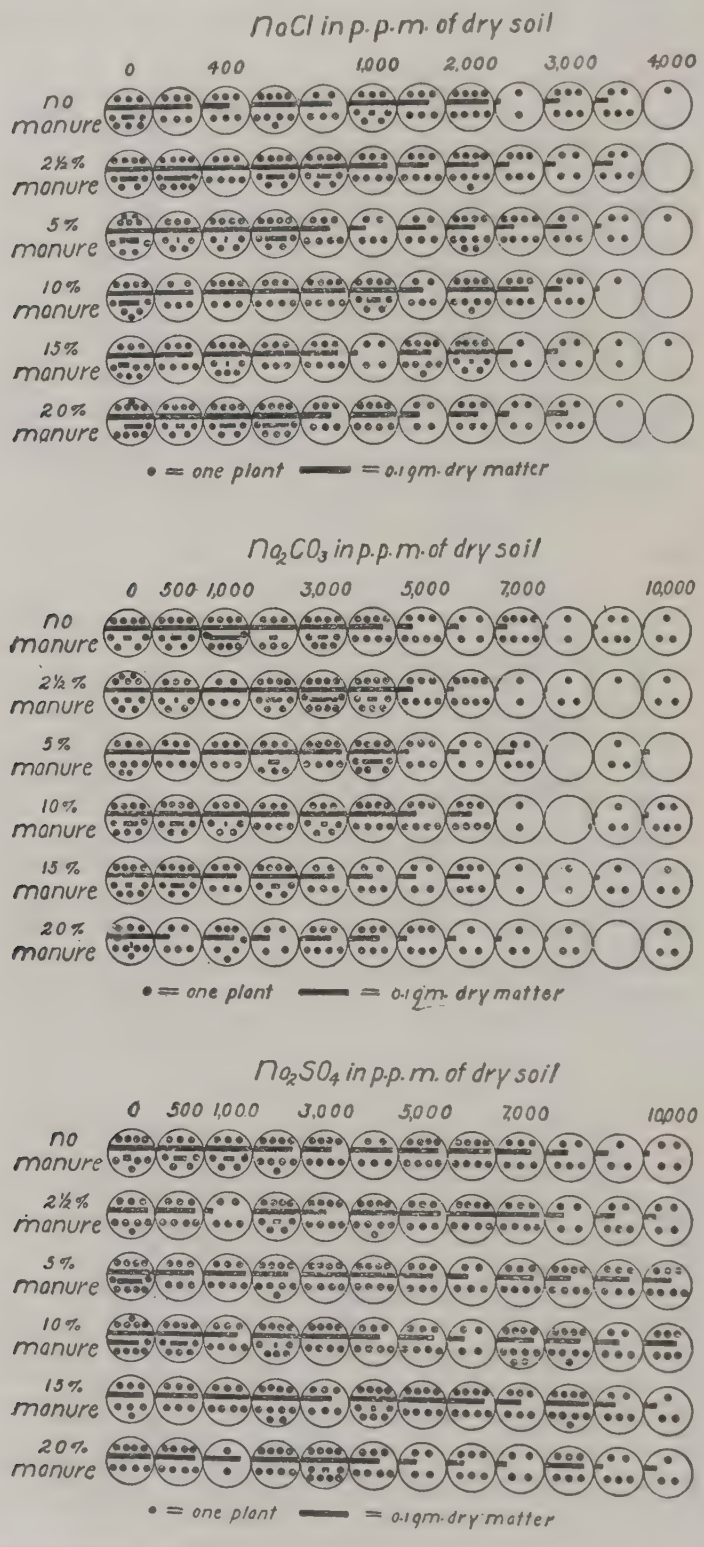

FIG. 16.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in loam with different amounts of manure containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations. Soils maintained at an equivalent moisture content.

tents and of fresh chaff, peat, and manure on loam. The peat used was a rotted woody and leafy material deposited by Logan River. The manure was fairly well rotted. The results are shown in figures 8 to 16.

Additional organic matter seemed to increase the tolerance of the soil for alkali if enough additional water was added to bring it up to an equivalent moisture content, but increased the toxicity if the extra

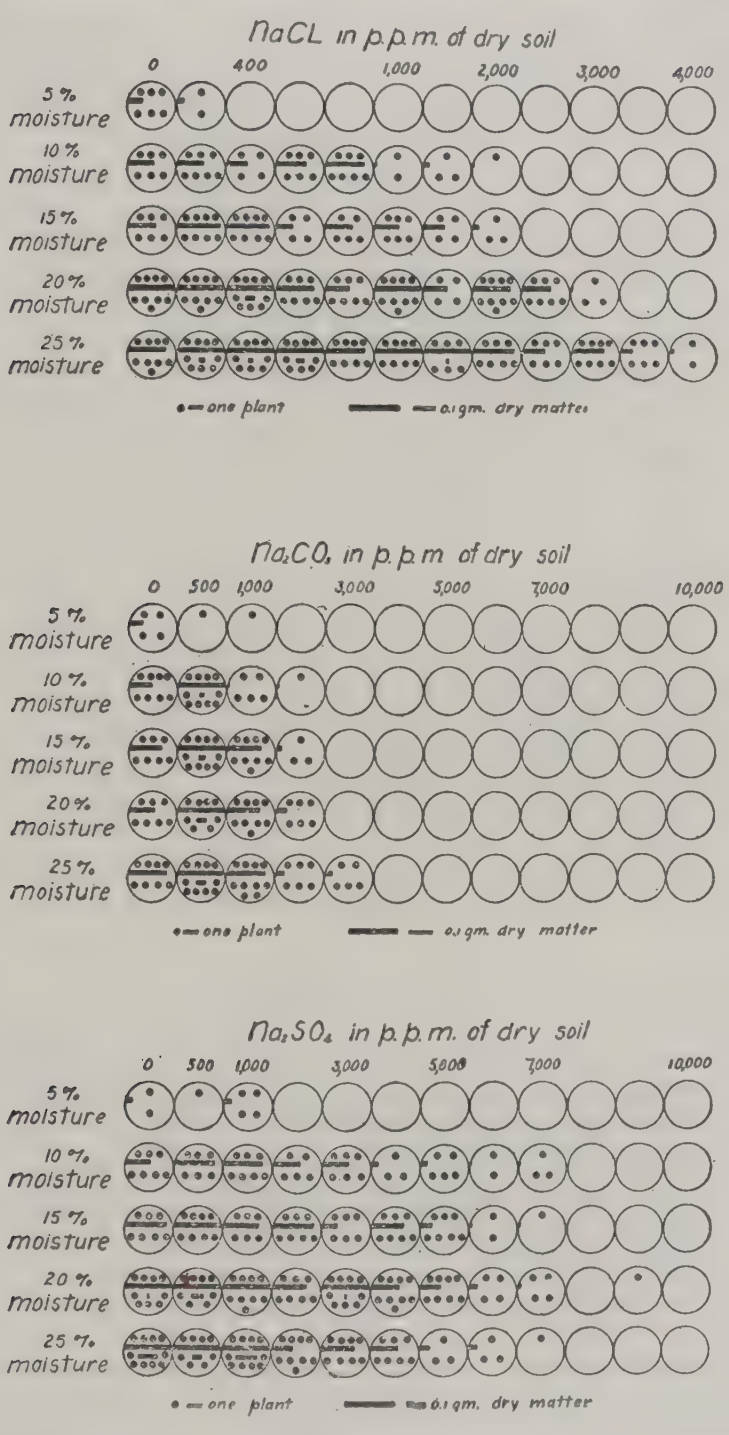

FIG. 17.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

moisture was not added. The greatest effect was produced by the peat probably because it was the finest and could be most thoroughly incorporated into the soil. The results were more noticeable in the case of the carbonates than in that of the two other salts.

EFFECT OF MOISTURE CONTENT

In order to test more completely the effect of different moisture contents on the toxicity of the alkali salts, a separate series of experiments was conducted using many of the soils used in the other experiments at different moisture contents. These varied between the lowest and highest extremes at which seeds could be made to germinate.

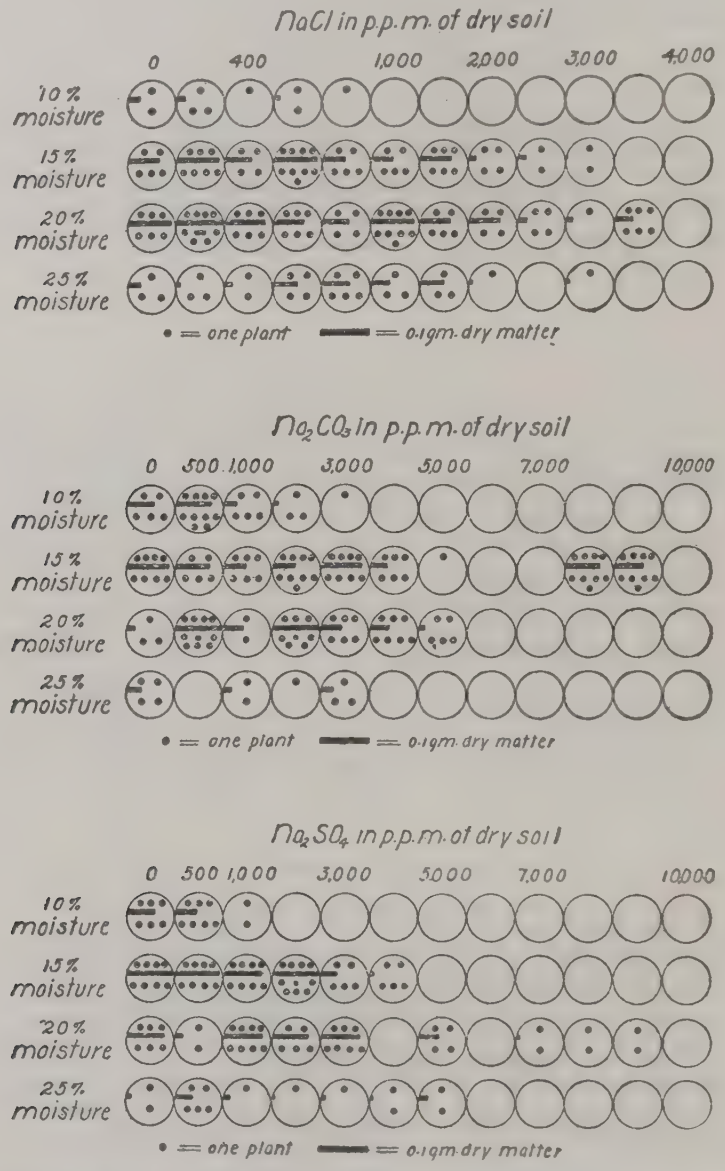

FIG. 18.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand and loam maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

The results which are given in figures 17 to 26 show that the resistance was increased with the increased moisture content up to the point of excessive moisture for plant growth. This was the most noticeable correlation observed in all these investigations. Two additional series were conducted on the plan of duplicating tumblers and using only four concentrations. In the first, the salt content of each set was the same

on the basis of the dry soil, but the moisture content varied. In the second, the soils were made up with different amounts of the same solution, the concentration being maintained by adding water as it was lost

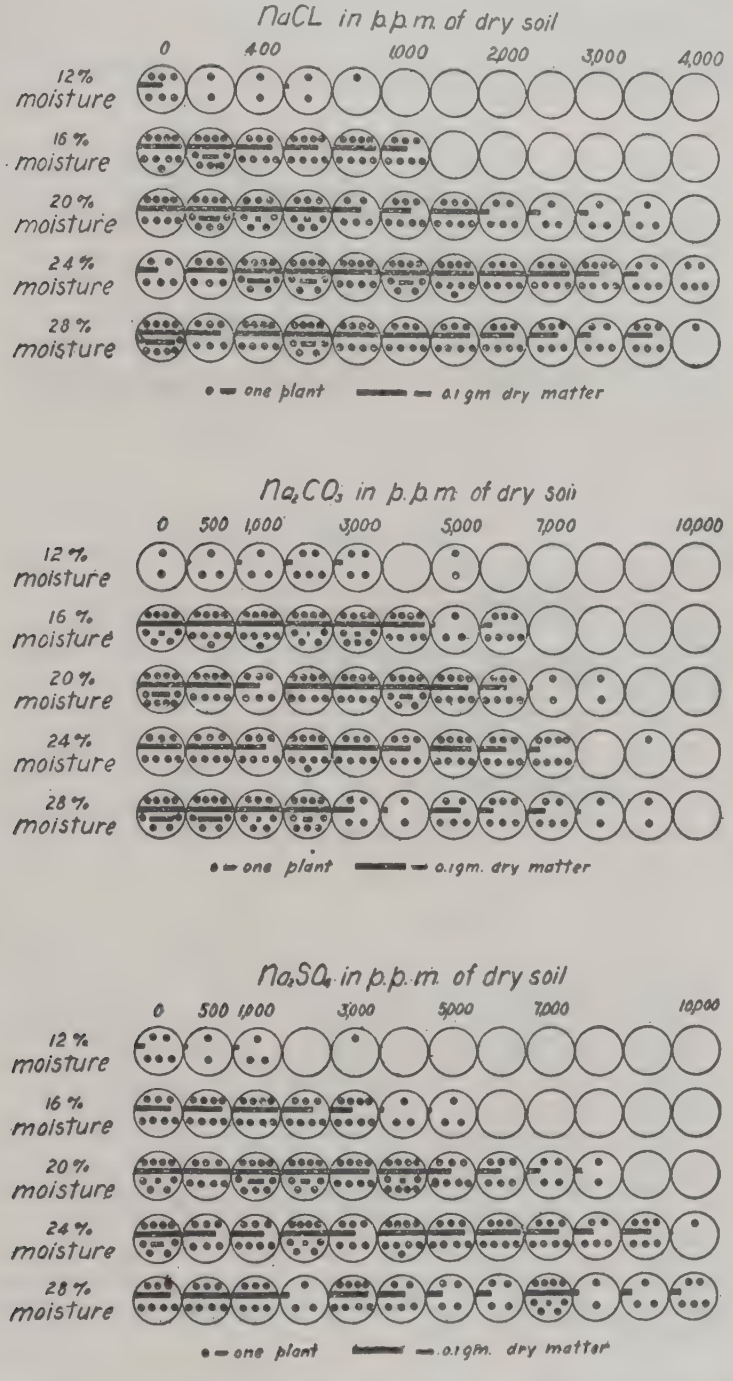

FIG. 19.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in loam maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations.

by evaporation. This would give theoretically the same concentration of salt in each case even though the percentage of moisture was varied. The results are given in Tables III and IV. These show that with soils having the same concentration of salts on the dry soil basis, the greatest

resistance was found at the highest moisture content consistent with good growth. In the second series, in which the theoretical concentration of the soil solution was the same, different results were observed. In the case of the chlorids and sulphates the toxicity was nearly proportional to the strength of the solution, the best growth otherwise being with the optimum moisture content. In the case of the carbonates, however, the

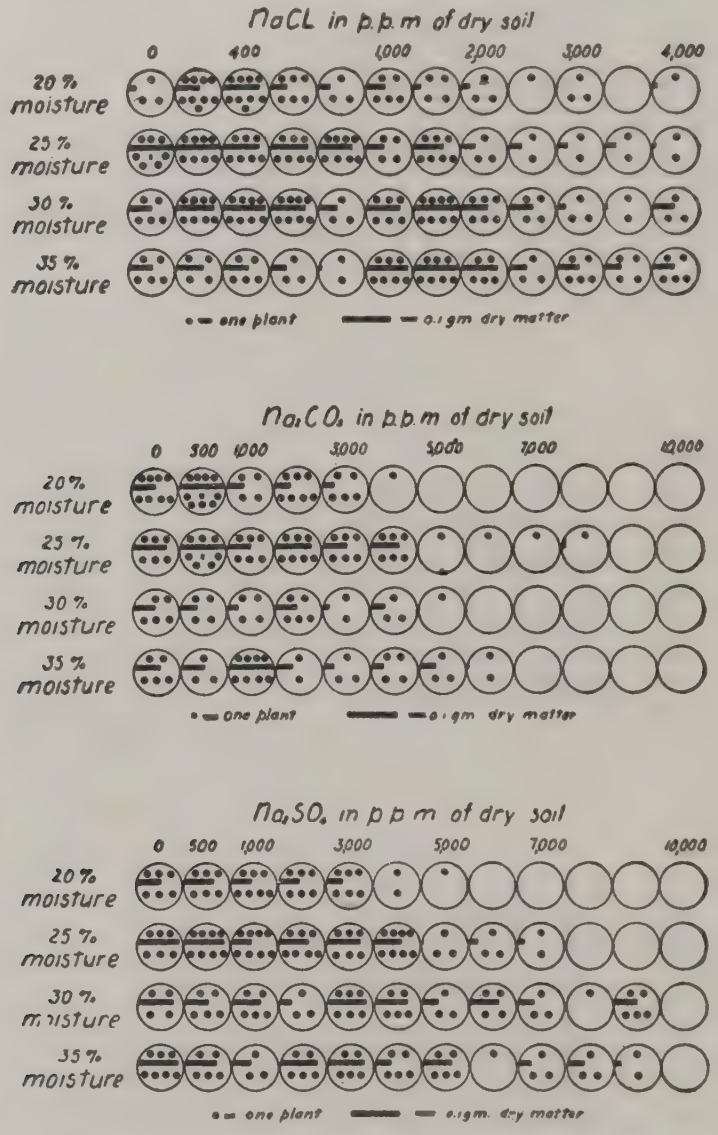

FIG. 20.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in loam and clay maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

greatest growth was where the least amount of solution, and consequently of salt, had been applied. This shows that in the case of the carbonates at least the concentration of the soil solution does not depend directly on the proportion of salt to soil, or on the proportion of salt to water, but on a combination of all these factors probably, depending on the relative absorptive powers of the soil for the salt and for water.

TABLE III.—*Average dry matter (in 0.0001 gm.) produced in loam soil maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations based on the dry weight of the soil*

Salt and concentration.	Dry matter produced.										Average.
Moisture in soil .....per cent..	12	14	16	18	20	22	24	26	28	30	
P. p. m.											
Control.....	300	866	1,222	1,315	1,383	1,489	1,190	1,502	1,241	1,049	1,156
SODIUM CHLORID.											
1,000.....	.....	595	521	1,218	1,143	1,186	1,368	1,260	782	737	881
2,000.....	.....	.....	17	168	575	843	1,083	1,070	891	563	521
3,000.....	.....	.....	.....	38	32	96	279	468	503	464	188
4,000.....	.....	.....	.....	.....	1	73	175	301	435	299	128
Average.....	.....	149	135	356	438	550	731	775	653	513	430
SODIUM CARBONATE.											
2,000.....	.....	343	752	1,087	1,013	992	1,069	965	927	624	777
4,000.....	.....	220	627	823	930	1,014	878	813	968	740	701
6,000.....	.....	.....	17	29	76	229	347	412	356	135	160
8,000.....	.....	.....	.....	.....	1	28	34	64	80	22	23
Average.....	.....	141	349	485	505	566	582	563	583	380	415
SODIUM SULPHATE.											
3,000.....	.....	77	245	808	806	1,141	1,261	918	722	843	682
6,000.....	.....	.....	9	56	504	265	709	1,049	948	359	390
9,000.....	.....	.....	.....	.....	.....	2	50	108	185	42	39
12,000.....	.....	.....	.....	.....	.....	.....	.....	.....	11	32	4
Average.....	.....	19	64	216	328	352	505	519	467	319	279

TABLE IV.—*Average dry matter (in 0.0001 gm.) produced in loam soil maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations based on the theoretical strength of the soil solution*

Salt and concentration.	Dry matter produced.										Average.
Moisture in soil .....per cent..	12	14	16	18	20	22	24	26	28	30	
P. p. m.											
Control.....	1,255	1,040	1,125	1,012	991	1,231	1,155	842	914	655	1,022
SODIUM CHLORID.											
5,000.....	652	908	903	943	1,191	1,190	927	764	697	129	830
10,000.....	299	706	1,033	1,007	925	1,095	908	821	464	12	727
15,000.....	251	389	558	618	610	737	899	523	554	292	543
20,000.....	313	281	196	292	173	131	259	322	311	112	239
Average.....	379	571	673	715	725	788	757	608	507	136	585
SODIUM CARBONATE.											
10,000.....	645	1,092	1,100	930	816	938	897	720	584	257	798
20,000.....	775	1,084	938	678	768	613	895	872	376	320	732
30,000.....	659	805	944	870	749	736	450	132	22	1	537
40,000.....	611	565	465	148	158	82	10	.....	.....	.....	204
Average.....	673	886	862	657	623	592	563	431	237	145	568
SODIUM SULPHATE.											
15,000.....	582	907	1,147	1,130	1,324	1,335	1,356	1,350	683	148	996
30,000.....	595	582	740	813	1,109	1,060	1,218	1,453	969	753	929
45,000.....	212	276	611	469	825	383	544	558	258	146	429
60,000.....	24	150	75	108	104	81	28	43	44	12	67
Average.....	355	479	643	630	840	715	787	851	488	265	605

EFFECT OF ADDED SALTS ON CONCENTRATION OF SOIL SOLUTION

In order to get some definite conclusions as to the actual concentration of the soil solution in these alkali soils under the conditions of the experi-

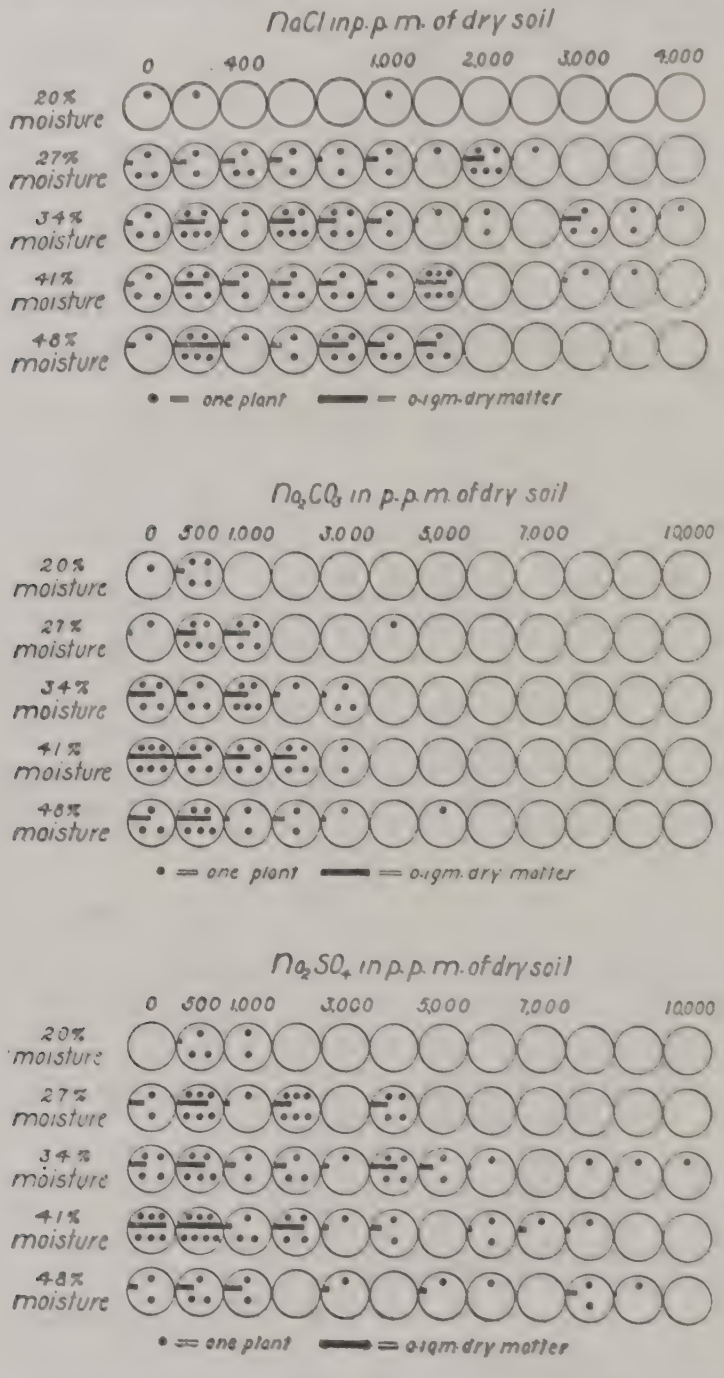

FIG. 21.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in clay maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

ment, the freezing-point method described by Bouyoucos and McCool[1] was used. The soils were dried and later made up to the moisture content

[1] BOUYOUCOS, G. J. and MCCOOL, M. M. THE FREEZING-POINT METHOD AS A NEW MEANS OF MEASURING THE CONCENTRATION OF THE SOIL SOLUTION DIRECTLY IN THE SOIL. Mich. Agr. Exp. Sta. Tech. Bul. 24, p. 592–631 2 fig. 1916.

used in the experiment. They were left standing in stoppered containers for forty-eight hours. It was found that no regular results could be obtained without this forty-eight-hour period of standing. The freezing point was then determined by the method referred to above. From solution experiments it was found that a gram-molecular solution of sodium chlorid froze at −3.56° C., sodium carbonate at −4.58° C.,

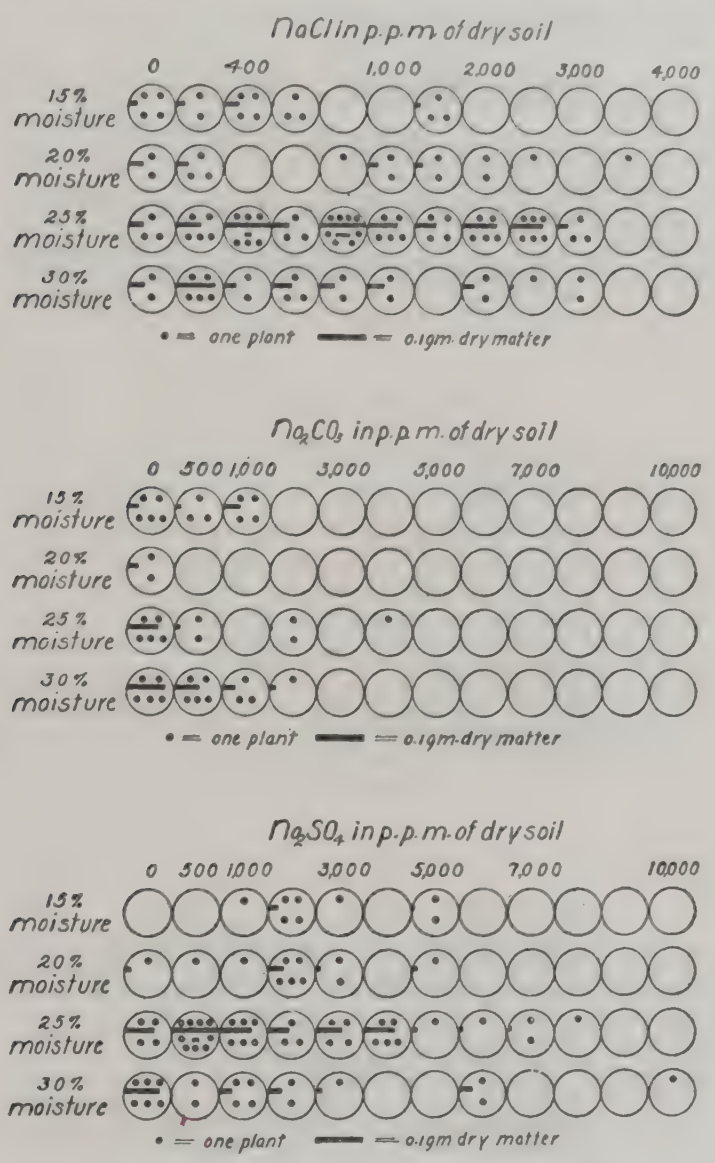

FIG. 22.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand and clay maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations.

and sodium sulphate at −4.37° C., the depression being directly proportional to the concentration. With these figures it was possible to calculate additional strength of the soil solution from the freezing point, assuming it contained only the salt added. From this and the theoretical strength, calculated from the amount of salt and water added, the relative adsorption of salt and of water could be calculated. It was found necessary to use a check with each series for comparison, since some

unknown factor caused a variation in all samples from day to day, but on each day the relation between the check and the treatment was consistent. It was found that the depression of the freezing point varies consistently with the concentration and that duplicate samples checked to

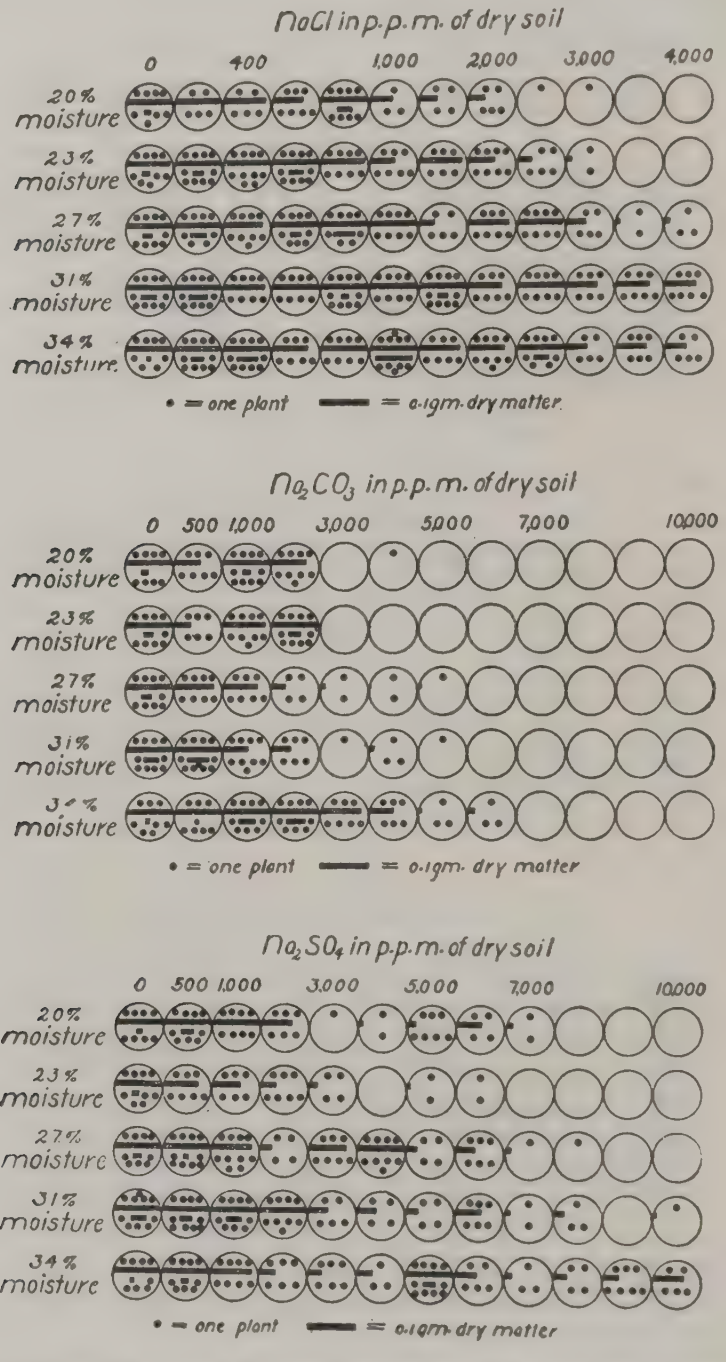

FIG. 23.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand and clay maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations.

within 0.01 or 0.02 degree. The freezing point of soils used in preparing figures 2, 3, and 4 was taken, and the results were averaged for the different content of each salt and for the different types of soil. The results are given in Tables V, VI, and VII, which show the quantity of the salt

added and the quantity that was determined by the freezing-point method to be actually in the soil solution. In the case of the added sodium chlorid it was noticeable that there is more salt shown than was added, which indicates either that there was more water than salt adsorbed by

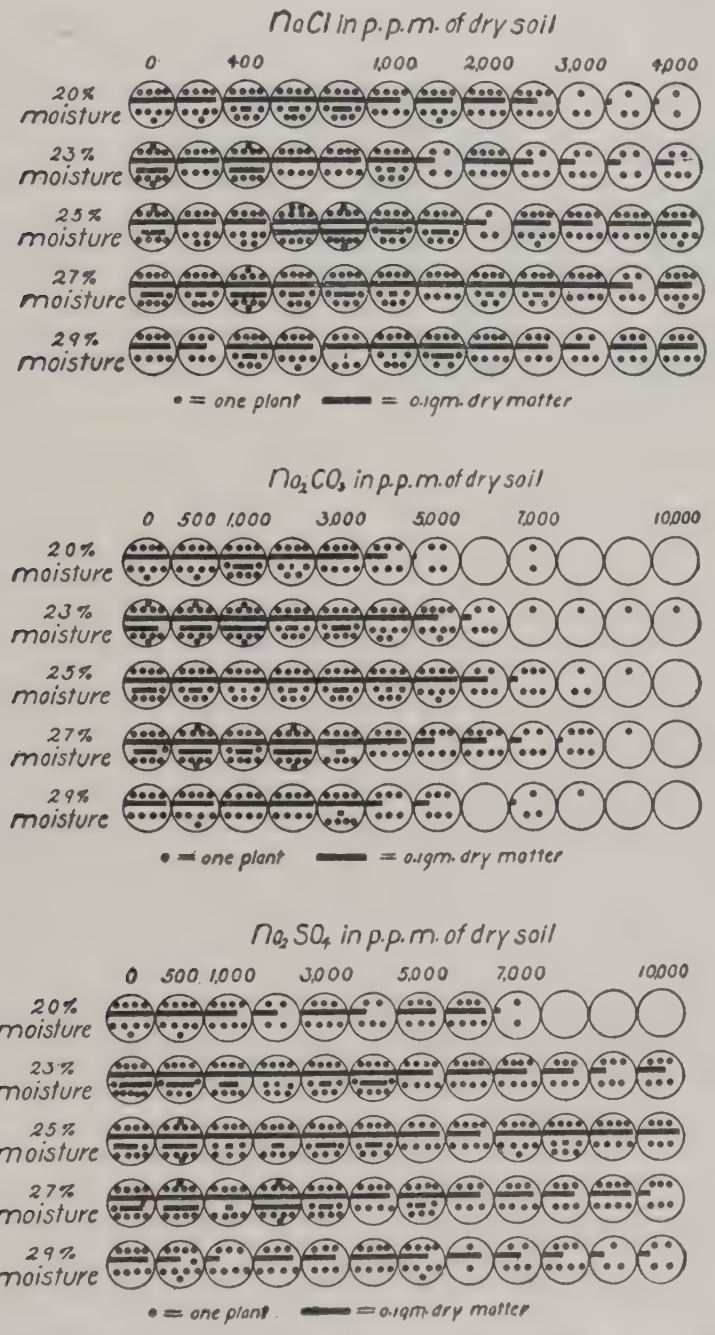

FIG. 24.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in sand and peat maintained at different concentrations and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

the soil or that other substances were brought into solution from the soil by adding the sodium chlorid. This effect reached a maximum at 2,500 p. p. m. of salt. There was no regular difference with the different soil types. In the case of the sulphates, apparently not all of the salt remained in solution except in the lowest concentrations. This was to

be expected from the large amount of the crystalline salt that appeared at the surface of the tumblers of higher concentration. The sodium carbonate shows the most interesting results. Over 80 per cent of the lowest concentration (500 p. p. m.) has disappeared from the solution, so far as its effect on the freezing point is concerned. It is also noticeable that the greatest adsorption occurred in the soils having the greatest amount of organic matter as was to be expected from our other results. The amount of the salt that could be detected in these soils by the ordinary method of water extraction and titration is shown in Table VIII, for comparison. The results agree remarkably well. This seems to show that the organic matter in the soil not only neutralizes the basicity of the carbonate but actually removes it from the solution altogether.

TABLE V.—*The increase in the concentration of the soil solution by adding sodium chlorid in various quantities to different soils. Increase in concentration determined by the depression of the freezing point and expressed as parts per million of sodium chlorid in dry soil*

Soil.	Concentration.										Average.
Salt added..p. p. m.	200	400	600	800	1,000	1,500	2,000	2,500	3,000	3,500	4,000	
Sand 90, loam 10...	231	560	889	1,251	1,844	2,865	3,886	5,302	6,389	8,662	8,958	3,712
Sand 80, loam 20...	527	650	1,284	1,515	1,844	2,569	4,677	6,224	6,587	6,916	9,090	3,808
Sand 70, loam 30...	99	856	1,120	1,284	1,844	2,601	3,359	3,491	5,730	6,488	5,269	2,922
Sand 60, loam 40...	33	296	626	1,087	1,219	2,009	3,425	4,446	5,072	6,225	7,443	2,898
Sand 40, loam 60...	395	650	790	1,021	2,437	2,470	3,326	3,952	4,512	5,105	5,368	2,730
Sand 30, loam 70...	263	593	725	988	1,251	1,976	2,734	3,195	4,150	4,742	5,269	2,353
Sand 20, loam 80...	231	560	650	1,087	1,284	1,844	2,668	3,326	3,557	4,380	5,171	2,251
Loam 90, clay 10...	33	132	461	692	889	1,614	2,569	2,832	3,293	3,820	4,183	1,865
Loam 80, clay 20....	231	428	428	560	955	1,647	2,075	2,602	3,195	3,425	4,380	1,811
Loam 70, clay 30...	66	231	494	692	757	1,284	1,482	2,075	2,700	3,557	3,655	1,545
Loam 60, clay 40...	33	165	362	494	922	1,350	1,811	2,437	2,964	3,293	3,985	1,620
Loam 40, clay 60...	132	296	296	659	955	1,515	2,470	2,799	3,063	3,853	4,084	1,829
Loam 30, clay 70...	165	395	527	889	988	1,482	1,811	2,437	3,030	3,557	3,623	1,719
Loam 20, clay 80...	33	132	362	659	790	1,416	2,042	1,943	3,260	3,293	2,668	1,509
Loam 10, clay 90...	395	626	790	922	1,284	1,581	2,305	2,898	3,656	3,491	4,018	1,997
Average.....	191	438	654	920	1,284	1,881	2,709	3,331	4,077	4,720	5,149	.....

TABLE VI.—*The increase in the concentration of the soil solution by adding sodium carbonate in various quantities to different soils. Increase in concentration determined by the depression of the freezing point and expressed as parts per million of sodium carbonate in dry soil*

Soil.	Concentration.										Average.
Salt added..p. p. m.	500	1,000	2,000	3,000	4,000	5,000	6,000	7,000	8,000	9,000	10,000	
Sand 90, loam 10...	93	1,072	1,864	3,262	5,685	6,852	9,361	9,600	12,256	12,256	13,514	6,892
Sand 80, loam 20...	47	280	699	1,869	3,500	5,219	5,965	8,947	10,019	10,205	10,347	5,190
Sand 70, loam 30...	47	559	559	1,481	3,122	3,821	4,707	6,990	8,901	9,273	10,066	4,503
Sand 60, loam 40...	47	186	652	932	1,911	2,886	3,821	4,613	6,291	8,295	9,413	3,549
Sand 40, loam 60...	93	233	470	839	1,198	1,455	2,470	3,776	6,291	6,617	4,893	2,576
Sand 30, loam 70...	.....	186	470	419	792	1,222	1,771	2,982	3,821	4,660	5,312	1,966
Sand 20, loam 80...	140	233	419	470	700	1,165	1,548	2,145	2,237	4,339	3,961	1,578
Loam 90, clay 10...	93	140	140	373	608	841	1,128	1,491	2,050	2,516	2,936	1,119
Loam 80, clay 20...	93	186	280	513	932	1,165	1,455	1,820	2,097	2,330	3,309	1,289
Loam 70, clay 30...	140	93	280	513	756	932	1,035	1,400	1,864	2,610	3,375	1,182
Loam 60, clay 40...	93	280	326	559	652	652	1,175	1,165	1,631	1,957	2,703	1,018
Loam 40, clay 60...	180	280	373	280	1,035	1,165	1,072	1,675	2,843	2,936	3,635	1,407
Loam 30, clay 70...	93	140	140	326	932	1,496	1,864	1,864	2,703	2,283	2,843	1,335
Loam 20, clay 80...	93	47	280	280	513	746	1,072	1,584	1,864	2,050	2,843	1,034
Loam 10, clay 90...	280	513	559	373	1,584	1,491	1,911	2,843	2,796	3,914	4,820	1,889
Average.....	102	295	501	832	1,595	2,074	2,690	3,526	4,511	5,082	5,578	.....

TABLE VII.—*The increase in the concentration of the soil solution by adding sodium sulphate in various quantities to different soils. Increase in concentration determined by the depression of the freezing point and expressed as parts per million of sodium sulphate in dry soil*

Soil.	Concentration.											Average.
Salt added..p.p.m.	500	1,000	2,000	3,000	4,000	5,000	6,000	7,000	8,000	9,000	10,000	
Sand 80, loam 20...	1,306	2,090	3,985	6,663	8,492	9,863	8,884	9,602	7,838	7,773	8,034	6,775
Sand 70, loam 30...	2,743	2,155	3,789	4,703	9,472	9,798	9,994	9,341	9,733	7,773	7,838	7,031
Sand 60, loam 40...	784	1,698	3,070	4,376	7,051	9,014	4,512	9,994	9,145	7,773	8,361	5,980
Sand 40, loam 60...	261	1,110	1,894	2,874	4,638	6,597	7,708	8,230	9,080	9,537	13,000	5,900
Sand 30, loam 70...	849	849	2,678	3,397	4,246	5,095	8,165	9,210	9,537	9,602	11,104	5,884
Sand 20, loam 80...	914	1,241	2,221	2,939	4,050	5,160	5,748	6,663	6,989	7,969	9,798	4,779
Loam 90, clay 10...	588	1,045	2,090	2,809	3,266	4,050	4,703	5,095	6,597	6,662	7,381	4,026
Loam 80, clay 20...	784	784	1,698	2,548	3,201	4,181	4,442	4,964	5,618	6,205	7,447	3,806
Loam 70, clay 30...	391	719	1,960	2,939	2,809	3,723	3,723	4,703	6,205	5,879	6,933	3,638
Loam 60, clay 40...	784	1,241	1,894	2,482	3,331	3,593	4,181	4,703	5,356	5,814	6,597	3,636
Loam 40, clay 60...	719	719	1,960	2,090	3,005	3,331	3,266	3,789	5,160	5,291	6,140	3,224
Loam 30, clay 70...	849	1,306	1,829	2,678	3,005	3,397	3,985	4,311	5,552	5,618	6,597	3,557
Loam 20, clay 80...	849	784	980	1,502	1,829	2,678	3,331	3,593	3,984	4,442	5,291	2,660
Loam 10, clay 90...	2,613	2,613	1,045	1,894	1,960	2,678	3,919	4,115	4,246	5,878	5,879	3,349
Average.....	1,031	1,314	2,221	3,135	4,311	5,233	5,468	6,308	6,788	6,872	7,885	.....

TABLE VIII.—*Amount of sodium carbonate that could be detected by water extraction in different soils to which it had been added in various quantities*

Soil.	Concentration.											Average.
Salt added..p.p.m.	500	1,000	2,000	3,000	4,000	5,000	6,000	7,000	8,000	9,000	10,000	
Sand 90, loam 10...	170	594	891	1,484	2,417	3,859	3,307	5,682	5,088	6,784	6,784	3,369
Sand 80, loam 20...	250	381	896	1,399	2,077	2,059	3,477	3,010	4,452	5,427	6,445	2,716
Sand 70, loam 30...	148	275	615	1,293	1,674	2,141	3,243	3,582	4,346	4,388	5,109	2,437
Sand 60, loam 40...	170	255	552	1,018	1,527	1,993	2,375	3,223	4,113	4,601	4,198	2,184
Sand 40, loam 60...	42	254	551	848	1,229	1,696	1,865	2,289	2,883	3,519	3,731	1,718
Sand 30, loam 70...	170	170	636	976	1,315	1,654	2,078	2,714	3,265	3,774	3,859	1,874
Sand 20, loam 80...	86	170	510	934	1,315	1,485	1,951	2,121	2,801	3,478	3,732	1,689
Loam 90, clay 10...	212	212	467	848	1,018	1,569	1,823	2,035	2,544	2,841	3,265	1,530
Loam 80, clay 20...	42	212	466	805	1,102	1,314	1,865	2,077	2,628	3,095	3,392	1,545
Loam 70, clay 30...	85	339	806	1,060	1,230	1,696	1,908	2,020	2,671	3,095	3,731	1,695
Loam 60, clay 40...	170	255	721	1,188	1,357	1,781	2,078	2,460	2,672	3,265	3,901	1,804
Loam 40, clay 60...	170	254	424	424	1,187	1,654	1,696	1,866	2,205	3,350	3,901	1,557
Loam 30, clay 70...	254	42	339	593	996	1,738	2,120	2,162	3,095	2,756	3,604	1,609
Loam 20, clay 80...	60	18	442	823	1,205	1,586	1,671	2,350	2,774	3,198	3,961	1,644
Loam 10, clay 90...	170	467	594	1,442	891	1,569	2,078	2,714	3,096	3,816	4,240	1,916
Average.....	147	260	594	1,009	1,369	1,853	2,236	2,687	3,242	3,826	4,257	.....

RELATIVE TOXICITY OF THE SALTS

Turning our attention to the relative toxicity of the three salts and averaging together the yields of dry matter from all tumblers in series having the same salt content, we obtain the results shown in figure 27, which shows the percentage of normal yield from all soil types and moisture contents for each concentration of each salt as added. It will be noticed that up to 1,000 p. p. m. all the salts are beneficial in the action, the carbonate being especially so. After 1,500 p. p. m. all the salts become increasingly toxic, the chlorid most so and the sulphate least, with the carbonate about halfway between.

Two very noticeable features of these results are that the chlorids average about twice as toxic as the carbonate on a basis of what was

added, and that there is notable discrepancy between the carbonate added to the soil and the amount as determined by water extraction. It would seem that these might to a certain extent be offered as a mutual

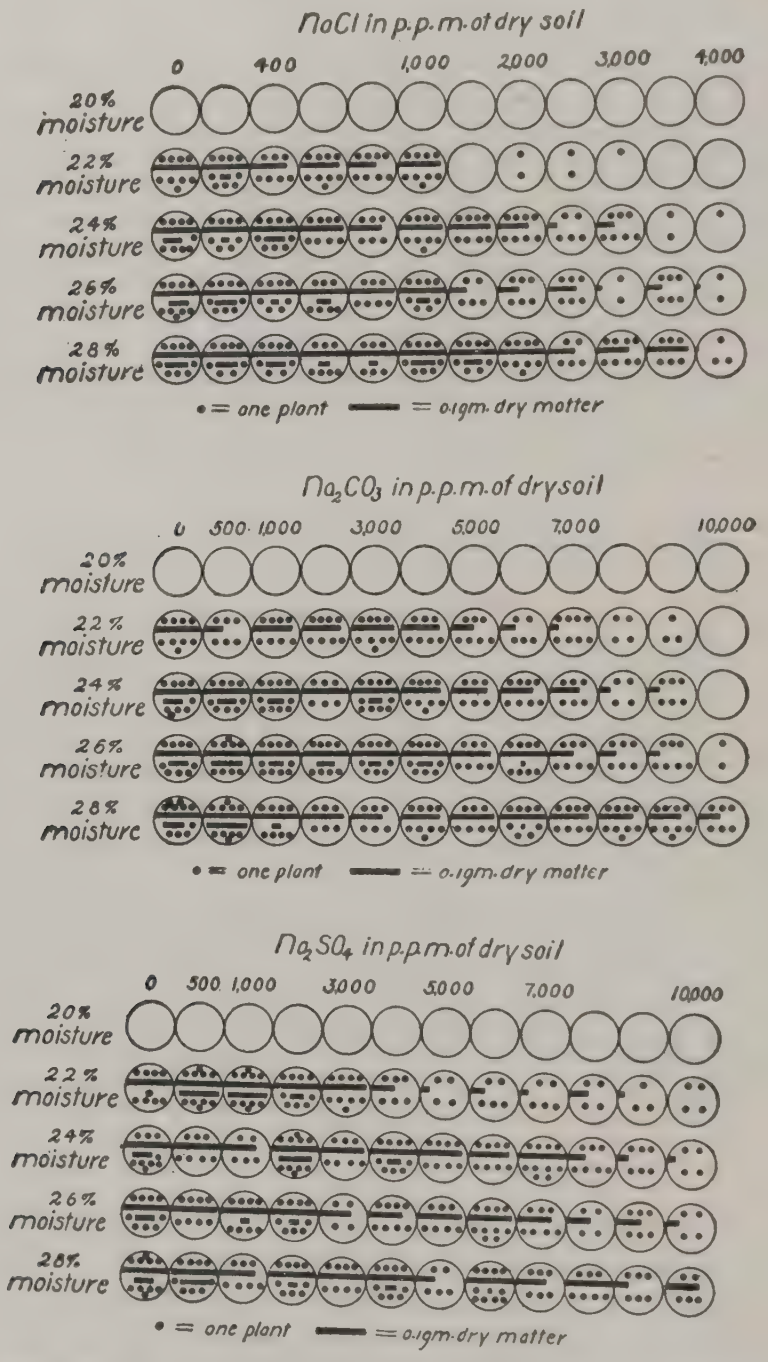

FIG. 25.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in loam and peat maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate added in various concentrations.

explanation of one another. Neither of these peculiarities was observed in our studies with sand.

Practically all of the results showing the carbonates to be more toxic than the chlorids have been obtained from studies in solutions and in sand cultures or from field studies where the salt has been determined

by water extractions. This extraction in experiments of this kind does not show all of the alkali carbonates that have been added. In averaging all the determinations it will be seen that less than half of the salt added was extracted. While this is not enough to change the results to the

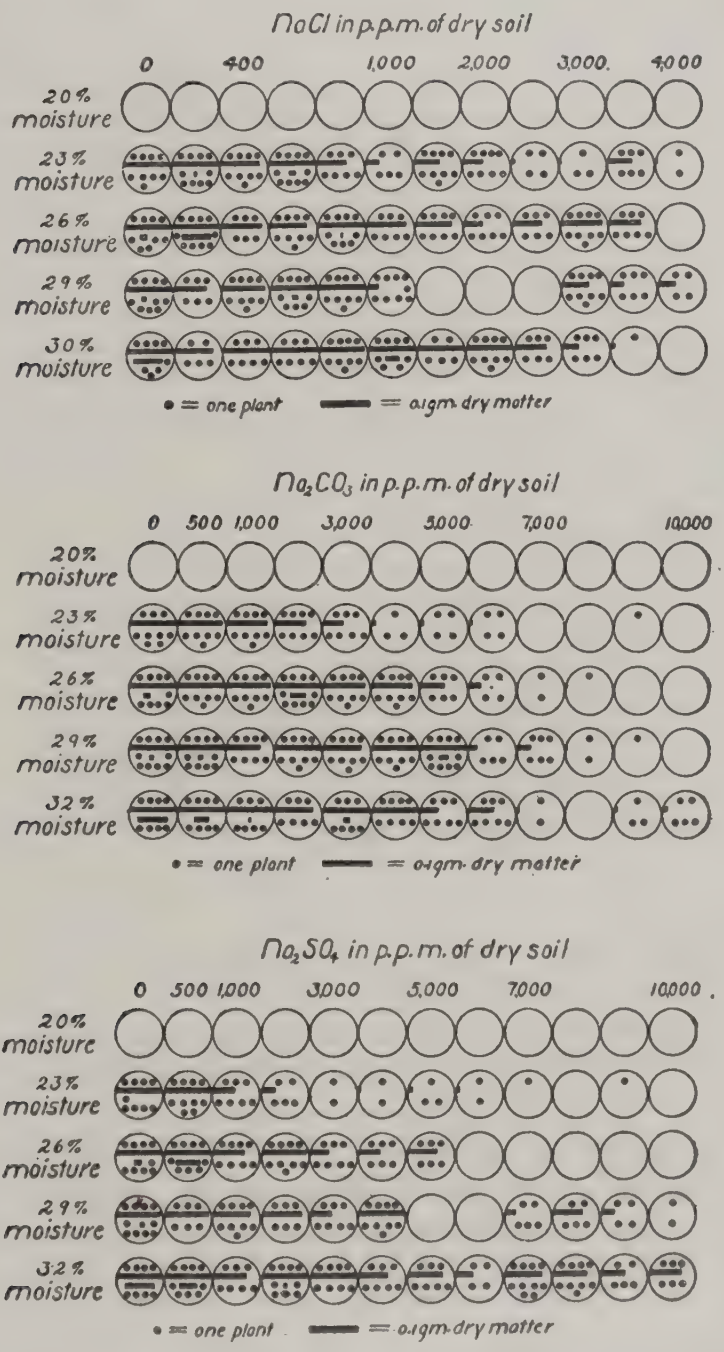

FIG. 26.—Diagram showing the number of wheat plants up and the dry matter produced in 21 days in loam and manure maintained at different moisture contents and containing sodium chlorid, sodium carbonate, and sodium sulphate in various concentrations.

customary statement that the carbonates are twice as toxic as the chlorids, it shows possibilities in this direction. The method of extracting was as follows: Fifty gm. of the oven-dry soil were added to 500 cc. of distilled water and stirred with a wooden paddle for five minutes. The

solution was allowed to settle for an hour and filtered through a Pasteur-Chamberland filter. Ten to fifty cc. of the clear solution were titrated with *N*/50 sulphuric acid, with methyl-orange as indicator and the results expressed as sodium carbonate. In cases where there was much organic matter in the soil the solution was very black, but by diluting with neutral water to a yellowish-brown color the end point could always be distinctly observed by one accustomed to making the titration. It was found that this method gave as nearly complete an extraction as

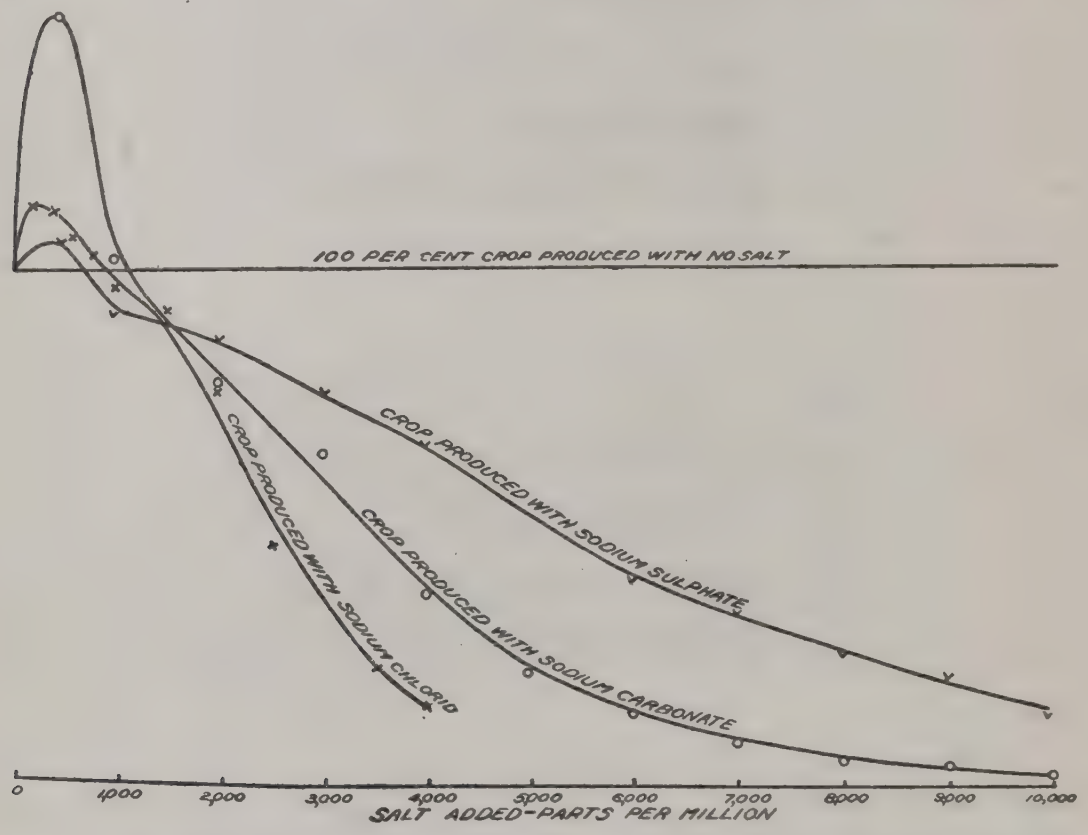

FIG. 27.—Graphs showing the percentage of normal yield of dry matter of wheat produced in 21 days with various concentrations of added sodium chlorid, sodium carbonate, and sodium sulphate. Average of all trials.

any method of cold-water extraction that we could find, but the solution was never complete. Since most field studies are made by water extractions, this may offer a partial explanation of the unusual nature of our results.

SUMMARY

(1) There is a great need for definite information regarding the toxicity of alkali salts in the soil.

(2) This paper reports about 12,000 determinations of the effect of alkali salts on plant germination and growth under different conditions.

(3) Size of particles of a sand independent of other factors does not seem appreciably to influence the toxicity of alkali.

(4) Loam soils are more tolerant of alkali than either sand or clay. The coarser loams are more tolerant than the finer at the same moisture content, but if the heavier loams are maintained at an equivalent moisture content they are more tolerant.

(5) Organic matter increases the resistance to alkali when the soil containing it is given sufficient moisture, but where present in large quantities organic matter decreases the resistance if the moisture supply is low.

(6) Increasing the moisture content of a soil up to the maximum that will produce good crops increases resistance to alkali.

(7) The toxicity of sodium chlorid and sodium sulphate seems to depend to quite an extent on the relation between concentration of salt and percentage of moisture present, while the toxicity of sodium carbonate is more largely affected by the presence of organic matter.

(8) Organic matter in the soil seems actually to remove sodium carbonate from the soil solution in large quantities.

(9) This probably explains why in experiments where sodium carbonate is added to a loam soil, it is less toxic than sodium chlorid, while in field studies where the salt is determined by analyses, and in solution and sand culture studies the sodium carbonate is more toxic.

Practical conclusions that may be drawn from these experiments are:

(1) Loam soils and soils with a high water-holding capacity may be successfully farmed at a higher alkali content than others.

(2) Soils in which alkali reduces crop yields should be kept as moist as is compatible with good plant growth.

(3) Manure, or other organic matter, should be beneficial to alkali soils, especially those high in carbonates.

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SOIL ACIDITY AS AFFECTED BY MOISTURE CONDITIONS OF THE SOIL

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INTRODUCTION

In an investigation of soils from tiled and untiled land it has been found that the drained was not as acid as the undrained soil (*2*).[1] To throw more light on the effect of moisture on soil acidity, five typical acid Indiana soils were selected for investigation under controlled moisture conditions: Soil A, a yellow silty clay from Jennings County; soil B, a whitish silt loam from Jennings County; soil C, a brown silt loam from Tippecanoe County; soil D, a black peaty sand from La Porte County; and soil E, a dark-brown peat from De Kalb County.

Equal quantities of each of these soils, the analyses of which are given in Table I, were kept in pots in the greenhouse at full water-holding capacity, at one-half water-holding capacity, and at one-fourth water-holding capacity. Other portions of each soil were taken when pots were filled and kept in an air-dry condition in the laboratory. The pots were of galvanized iron of 770 cubic inches capacity, heavily coated on the inside with paraffin. The soil in each pot was kept under the desired moisture conditions by weighing them two or three times each week and replenishing the evaporated moisture with pure distilled water. The water-holding capacity of each soil was determined by placing a perforated bottom cylinder containing about 100 gms. of loose dry soil in a vessel of water and allowing the sample to become thoroughly saturated, then weighing. The soils fully saturated with water soon became more or less puddled, and the moisture determinations of these samples taken at the end of the test showed less than the calculated percentage of water. The moisture determinations of the samples with one-fourth and one-half water-holding capacity were approximately the same as the calculated percentages. The soils were placed in the pots February 27, 1917, and the experiment was continued for one year, after which they were sampled by means of a soil tube, taking a vertical core of soil to the full depth of the pot. Each sample was thoroughly mixed and divided

[1] Reference is made by number (italic) to "Literature cited," p. 329.

into two portions, one of which was sealed in an air-tight jar. The other was spread out and air-dried in the laboratory. Moisture was determined in both the moist and the air-dry samples. Acidity was determined by the Hopkins potassium-nitrate method,[1] by the C. H. Jones calcium-acetate method (*4*), and by the ethyl-acetate method (*1*). The results of these determinations are given in Table II. In making the acidity determinations, the moisture contents of the soils were taken into consideration so that the proportions of dry soil to water and reagent used in making the tests were the same for all samples of both wet and air-dry soils.

TABLE I.—*Analyses of soils used*

Determined.[a]	A.	B.	C.	D.	E.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Volatile matter	3.57	3.92	7.45	10.13	85.20
Potassium oxid (K_2O)	.27	.25	.40	.21	.17
Calcium oxid (CaO)	.18	.17	.37	.10	.46
Magnesium oxid (MgO)	.40	.24	.61	.23	.20
Manganese oxid (Mn_3O_4)	.08	.04	.17	.04	.02
Ferric oxid (Fe_2O_3)	3.68	1.28	3.04	1.04	.48
Aluminium oxid (Al_2O_3)	4.68	2.80	4.57	3.09	.85
Phosphorus oxid (P_2O_5)	.05	.06	.15	.10	.13
Sulphate (SO_3)	.12	.10	.16	.11	.31
Residue	87.76	92.57	83.42	85.50	12.31
Nitrogen	.07	.12	.22	.40	2.04
Humus (acid)[b]	.73	1.31	2.25	5.72	50.19
Humus[c]	.70	1.17	3.13	4.06	52.00
Hygroscopic moisture	1.50	1.30	1.84	1.90	8.38
Acidity:					
Potassium-nitrate method[d] .lb.	5,460	2,220	460	2,520	5,080
Calcium-acetate method[d] .lb.	5,875	4,875	8,125	10,625	69,750
Water-holding capacity[e]	48.6	48.7	55.1	67.1	200.0

[a] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted, 1912.

[b] Ammonia soluble without previous washing with dilute hydrochloric acid.

[c] Washed with hydrochloric acid, digested with ammonia, filtered, and refiltered till clear.

[d] Pounds of calcium carbonate required to neutralize 2,000,000 pounds of soil.

[e] Water-holding capacity in grams of water per 100 gm. of dry soil.

In studying the results given in Table II, the following rather striking points are noted:

In each soil the degree of acidity, as indicated by all the methods used, was greater when the soil was held at one-half water-holding capacity than when it was held at one-fourth water-holding capacity.

All the soils which had been carried at one-half water-holding capacity were more acid than they were at the beginning of the experiment. This is in accord with the results obtained by Noyes and Yoder (*5*).

The soils high in organic matter gave greater acidity when held at full water capacity than when kept one-half saturated with water. The soils low in organic matter gave a lower degree of acidity when kept at full water than when kept at one-half water-holding capacity.

[1] WILEY, H. W., ed. OP. CIT.

TABLE II.—*Relative acidities of soils with different moisture conditions, and changes due to drying*

Soil.	Moisture treatment.	Potassium-nitrate method.[a]			Calcium-acetate method.[a]			Ethyl-acetate method.[b]		
		Moist.	Dried.	Change.	Moist.	Dried.	Change.	Moist.	Dried.	Change.
A[c]	Start	.....	5,460	.....	.....	5,875	.....	.....	.....	.....
	End	.....	4,160	.....	.....	7,000	.....	.....	.....	.....
	½ water	6,165	6,150	− 15	9,500	9,000	− 500	0.0011	0.0006	−0.0005
	Full water	3,750	3,475	− 275	7,000	6,250	− 750	.0009	.0006	− .0003
B	Start	.....	2,220	.....	.....	4,875	.....	.....	.....	.....
	End	.....	1,720	.....	.....	5,000	.....	.....	.....	.....
	¼ water	3,075	3,100	+ 25	6,250	6,250	0	.0034	.0011	− .0023
	½ water	3,625	3,725	+ 100	7,500	7,000	− 500	.0038	.0017	− .0021
	Full water	2,200	1,775	− 425	6,500	5,500	−1,000	.0026	.0013	− .0013
C	Start	.....	460	.....	.....	8,125	.....	.....	.....	.....
	End	.....	360	.....	.....	7,500	.....	.....	.....	.....
	¼ water	725	650	− 75	8,000	9,000	+1,000	.0027	.0013	− .0014
	½ water	1,000	975	− 25	8,750	10,000	+1,250	.0029	.0013	− .0016
	Full water	2,150	450	−1,700	6,250	5,000	−1,250	.0025	.0011	− .0014
D	Start	.....	2,520	.....	.....	10,625	.....	.....	.....	.....
	End	.....	2,360	.....	.....	9,500	.....	.....	.....	.....
	¼ water	2,875	3,125	+ 250	10,750	13,000	+2,250	.0081	.0050	− .0031
	½ water	3,300	3,625	+ 325	11,500	12,500	+1,000	.0090	.0050	− .0040
	Full water	3,700	1,850	−1,850	12,000	9,500	−2,500	.0090	.0055	− .0035
E	Start	.....	5,080	.....	.....	69,750	.....	.....	.....	.....
	End	.....	5,000	.....	.....	66,000	.....	.....	.....	.....
	¼ water	3,950	4,000	+ 50	63,500	63,500	0	.0081	.0064	− .0017
	½ water	5,675	5,400	− 275	72,500	65,000	−7,500	.0103	.0064	− .0039
	Full water	8,000	6,720	−1,280	76,000	69,500	−6,500	.0120	.0060	− .0060

[a] Results expressed in pounds of calcium carbonate required for 2,000,000 pounds soil.

[b] Ten gm. of soil were placed in 100 cc. of pure 5 per cent ethyl-acetate solution and shaken at frequent intervals. The solutions were kept in a thermostat at 27° C. for 24 hours. Then 10 cc. of the supernatant liquid was removed and titrated with *N*/20 sodium hydroxid, phenolphthalein being used as the indicator. The figures reported are the constants calculated from the formula $K=1/t \log a/a-x$, where a equals grams of ethyl acetate at start and $a-x$ equals grams of ethyl acetate left at t (one day). The constant for *N*/1,000 acetic acid carried under like conditions was 0.0004 and for *N*/1,000 nitric acid it was 0.0039. These constants are relative only. Autocatalysis was noted in longer time reactions, but this factor has been ignored in the calculations reported.

[c] There was not enough of soil A, and the one-fourth water-holding capacity pot was omitted.

When the samples of moist soil taken at the close of the experiment were air-dried, those samples that had been kept saturated decreased markedly in acidity according to all methods used. When the samples kept at one-fourth and one-half water capacities were air-dried, all deceased in acidity according to the ethyl-acetate method, but the Hopkins and Jones methods gave both increases and decreases in acidity. While the acidity was generally decreased when the soils were air-dried, the degree of acidity equilibrium reached varied to a large extent, owing to the condition of equilibrium caused by the variation in moisture content at which the soil had been held. For instance, while undried soils containing much organic matter gave a higher acidity at full water than at half water capacity, these same soils when air-dried gave a much lower acidity after being held at full water than when held at one-half water-holding capacities. This reversal in order of acidity is not so apparent with soils low in organic matter.

Preliminary tests were made on the soils from samples taken nine months from the beginning. The results obtained with the samples from the pots of fully saturated soil show the extreme sensitiveness of

soils to slight variations in moisture. The sample of fully saturated soil C, taken at nine months, lost some moisture before it was determined, in which condition it had 27.2 per cent of water and 400 pounds' acidity by the potassium-nitrate method. Three months later a sample of soil from the same pot had an acidity by the same method of 2,150 pounds with 30.6 per cent of water. The soil from this pot showed but a slight trace of iron in the potassium-nitrate extract with 27.2 per cent of water and a very large amount with 30.6 per cent of water. This increase in acidity and of soluble iron appears to be due to the fully satuated condition rather than the longer time elapsed.

The relative acidities of the various soils, high or low in organic matter, gave quite wide variations with the different methods. In general, the potassium-nitrate method measures mineral acidity, owing to acid-reacting silicates, and to a less degree it measures acid organic matter in the soil. The calcium-acetate method, on the other hand, measures acidity due to acid-reacting silicates, and in addition it responds readily to acid organic matter. With soils high in organic matter the results due to this method are influenced by organic matter more than by acid silicates. Water-soluble acidity only slightly affects the results shown by either the potassium-nitrate or the calcium-acetate methods. The results shown by the ethyl-acetate test are very largely in proportion to the strength of the water-soluble acidity of the soil. These results would be affected by nitrates, sulphates, or chlorids of aluminium, iron, and to a slight degree by manganese salts; also by any soluble acid reacting organic matter. Pure silicates which show a very high acidity by other methods and which are not soluble in water do not affect ethyl acetate at all (*1*).

In titrating the potassium-nitrate acidity determinations, quite distinct differences were noted in the character of the precipitates formed. In order to study this point, determinations were made of the metals which had been dissolved in the reactions between the normal potassium-nitrate solution and the soil. Table III gives the bases and soluble silica found in 100 cc. of potassium-nitrate extract from both the wet and the air-dried soils; also the increases or decreases of soluble matter found on air drying the soil samples. These results show that considerable iron was made soluble when the soil was kept fully saturated. This soluble iron was apparently all in the ferrous state. After the soils were air-dry, the iron was very quickly and almost completely oxidized, as the air-dry soil showed but little soluble iron. This chemical change in the condition of the iron undoubtedly accounts for a large part of the decrease in acidity caused by drying the fully saturated samples. Soluble iron is seldom found in soil solutions in very large amounts, which is undoubtedly due to the fact that the usual procedure in preparing soil samples for analysis is first to air-dry them, allowing ample opportunity for the oxidation of the iron.

TABLE III.—*Soluble oxids in normal potassium-nitrate extract of soils with changes due to drying*

[Results expressed as grams of oxids per 100 cc. of extract from acidity determinations]

Soil.	Moisture treatment.	Silicic oxid.			Aluminium oxid.			Ferric oxid.		
		Wet.	Dry.	Change.	Wet.	Dry.	Change.	Wet.	Dry.	Change.
A	½ water	0.0062	0.0065	+0.0003	0.0421	0.0382	−0.0039	0.0020	0.0010	−0.0010
	Full water	.0045	.0048	+ .0003	.0179	.0180	+ .0001	.0040	.0020	− .0020
B	¼ water	.0049	.0039	− .0010	.0207	.0250	+ .0043	.0020	.0020	.0000
	½ water	.0045	.0057	+ .0012	.0210	.0236	+ .0026	.0020	.0010	− .0010
	Full water	.0046	.0024	− .0022	.0079	.0078	− .0001	.0140	.0030	− .0110
C	¼ water	.0032	.0036	+ .0004	.0075	.0070	− .0005	.0020	.0010	− .0010
	½ water	.0037	.0066	+ .0029	.0084	.0056	− .0028	.0020	.0010	− .0010
	Full water	.0034	.0032	− .0002	.0072	.0054	− .0018	.0240	.0010	− .0230
D	¼ water	.0050	.0054	+ .0004	.0193	.0258	+ .0065	.0020	.0010	− .0010
	½ water	.0056	.0041	− .0015	.0234	.0304	+ .0070	.0020	.0010	− .0010
	Full water	.0049	.0030	− .0019	.0225	.0068	− .0157	.0180	.0040	− .0140
	¼ water	.0053	.0021	− .0032	.0210	.0136	− .0074	.0020	.0020	.0000
	½ water	.0058	.0030	− .0028	.0199	.0166	− .0033	.0020	.0020	.0000
	Full water	.0090	.0040	− .0050	.0207	.0138	− .0069	.0380	.0040	− .0340

Soil.	Moisture treatment.	Manganese oxid.			Calcium oxid.			Magnesium oxid.		
		Wet.	Dry.	Change.	Wet.	Dry.	Change.	Wet.	Dry.	Change.
A	½ water	0.0086	0.0064	−0.0022	0.0370	0.0408	+0.0038	0.0116	0.0124	+0.0008
	Full water	.0200	.0200	.0000	.0288	.0488	+ .0200	.0141	.0135	− .0006
B	¼ water	.0035	.0035	.0000	.0350	.0380	+ .0030	.0070	.0069	− .0001
	½ water	.0034	.0030	− .0004	.0316	.0336	+ .0020	.0065	.0056	− .0009
	Full water	.0069	.0078	+ .0009	.0387	.0448	+ .0061	.0087	.0066	− .0021
C	¼ water	.0080	.0080	.0000	.1123	.1298	+ .0175	.0105	.0133	+ .0028
	½ water	.0040	.0060	+ .0020	.1251	.1302	+ .0051	.0130	.0136	+ .0006
	Full water	.0324	.0356	+ .0032	.0814	.1264	+ .0450	.0134	.0152	+ .0018
D	¼ water	.0040	.0040	.0000	.0122	.0128	+ .0006	.0042	.0041	− .0001
	½ water	.0020	.0020	.0000	.0066	.0094	+ .0028	.0021	.0028	+ .0007
	Full water	.0040	.0040	.0000	.0143	.0212	+ .0069	.0051	.0041	− .0010
	¼ water	.0052	.0034	− .0018	.0626	.0612	− .0014	.0086	.0054	− .0032
	½ water	.0056	.0034	− .0022	.0541	.0376	− .0165	.0072	.0046	− .0026
	Full water	.0034	.0036	+ .0002	.0504	.0464	− .0040	.0086	.0055	− .0031

There was a great difference in the amounts of manganese found in some of the soils. The manganese, like the iron, appears to have been very largely reduced and made soluble by saturating the soil with water and excluding the air. In soils A and C over one-half the total soil manganese was dissolved by the potassium-nitrate solution. Unlike iron, the manganese did not rapidly oxidize upon air-drying the soil. Undoubtedly, under proper conditions, oxidation of manganese takes place, although much less rapidly than that of iron. In view of the wide variations between the manganese results, new solutions were prepared, and the gravimetric determinations were checked and confirmed by means of the Volhard volumetric and the lead-peroxid colorimetric methods.

The soluble aluminium decreased when the fully saturated soils containing much organic matter were dried. With soils B and D, one-fourth and one-half saturated with water, the soluble aluminium increased on air-drying. This is in accord with the acidity, which likewise increased when these soils were dried. Different investigators have endeavored to correlate the amounts of soluble aluminium and iron with the degree of acidity as obtained with the potassium-nitrate method. The results given in Tables II and III show a certain correlation along this line, but it is very apparent that the titrated acidity can not be entirely explained on the basis of the amount of potassium-nitrate soluble aluminium and iron. This acidity is apparently partly due to soluble acid organic compounds in addition to the iron and aluminium compounds.

The amount of calcium in solution varied to a large degree in inverse relation to the aluminium and iron. In all the soils, except the peat (E), the solutions from the air-dried soils contained more calcium than did those from the undried soils. Magnesium and soluble silica showed no striking variations due to the varied moisture conditions.

The changes shown in the degrees of acidity and also the differences in the amounts of soluble bases occurring when the soil samples are air-dried indicate the importance of further study of soils and soil reactions on samples which are kept under field moisture conditions. Some of the reactions which occur when soils are dried are apparently very rapid and so slowly reversible that the composition of dried soils may be quite different from that of field soils.

MOISTURE REACTIONS OF ACID SOILS

It has been noted by different investigators that carbonated water will extract from a mineral soil a solution that on boiling to drive off the carbon dioxid will be alkaline to phenolphalein. This fact can hardly be taken as proof that the soil moisture is not acid or that the soil acidity has been regulated by the formation of carbonates. Such an extraction of bases by an acid is, of course, to be expected from the laws of chemistry, but it does not tell in what state of equilibrium the soil bases may have been before they were extracted. Recent researches would indicate that the soil moisture of acid soils is distinctly acid and not basic in reaction. Gillespie (5), working with the hydrogen electrode, has found that solutions of acid soils are distinctly acid in reaction. Sharp and Hoagland (6) likewise found that there is an excess of hydrogen ions in solutions of acid soils. In addition they say:

> Soils containing calcium in equilibrium with HCO_3 and CO_2 have a very slightly alkaline reaction

and

> The figure for $Ca(HCO_3)_2$ is almost identical with those obtained for the alkaline soils.

Truog (7) and Meacham found that the reaction of the plant sap of a number of agricultural plants was always acid. As most plants will grow in slightly acid soils and in slightly acid water cultures, it does not seem necessary nor even possible that in such cases calcium is first transformed into bicarbonate before it is assimilated. As a result of varying the moisture conditions of acid soils it is very evident from the results given in Tables II and III that chemical reactions take place as different conditions of equilibrium due to moisture and aeration are established. These reactions are in the nature of reduction, oxidation, and hydrolysis as well as interactions following the law of mass action between compounds which may be made chemically active. All of these chemical changes in the soil cause variations in the degree of water-soluble acidity, as shown by the ethyl-acetate reaction as well as of the less-soluble acidity which is shown by the soluble-salt methods. These changes would no doubt also affect the toxicity of acids and other compounds in the soil. For instance, it may be readily seen that the oxidation of iron in the soil from the ferrous to the ferric condition would reduce toxicity as well as acidity. Acid marsh soils containing much iron are unproductive until some time after they have been drained. These soils when properly drained become quite red from oxidized iron, in which condition they are much more productive. This fact is a matter of common knowledge among observant farmers in such regions. It is undoubtedly true that soil processes in which carbon dioxid is evolved also produce material changes in soil acidity (5). Nitrification also increases water-solubility acidity. These biological reactions, of course, depend materially upon soil-moisture conditions.

FACTORS AFFECTING SOIL ACIDITY

Primarily soil acidity is due to an excess of acid-reacting compounds, or, in other words, to a deficiency of bases. This deficiency of bases is caused to a large extent by the leaching of the calcium and magnesium in the drainage waters. A lesser factor is the removal of mineral bases by crops. Under ordinary conditions of decay the carbonaceous and nitrogenous matter of plants takes on an acid character, tending to neutralize bases in the soil. The acidity of peat soils is very largely organic, as shown by the fact that the ash of the most acid peat is basic in reaction. In mineral soils there is an enormous excess of silicic oxid. This silicic oxid when free is insoluble and inactive as an acid; but it is potentially acid, and under humid conditions tends to form chemically-active acid-reacting silicates of iron, aluminium, and manganese. The degree of acidity of aluminium silicate is in proportion to the ratio of silicic oxid and aluminium oxid and also to the amount of combined water the silicate contains (1). Everything else being equal, the more water there is in the silicate, the more active it is chemically and the more acid it is in reaction. The measurable acidity of the organic matter

of soils is also increased in the presence of an excess of water, as indicated from the results obtained with soil E. Drainage conditions will modify the acidity of either an organic or inorganic soil, and this will have an effect on soil fertility. Of course with soils well supplied with calcium and magnesium, poorly-drained soils would not become acid until a part of the bases were dissolved and washed away.

SUMMARY

(1) Five different types of acid soils were kept under different moisture conditions in pots in the greenhouse for one year. Portions of soil were kept one-fourth saturated, one-half saturated, and fully saturated; also in an air-dry condition.

(2) Acidity determinations were made by the Hopkins potassium-nitrate method, the C. H. Jones calcium-acetate method, and the ethyl-acetate method. Samples of soil from each pot were tested for acidity both in the moist and in the air-dried condition. The potassium-nitrate extracts were analyzed.

(3) The degree of soil acidity measured by the different methods varied with different moisture conditions.

(4) With each soil and each method used the samples which had been kept half-saturated were higher in acidity than they were at the start of the experiment. The acidity of the half-saturated soils was greater than the acidity of the fourth-saturated soils.

(5) The soils high in organic matter showed the greatest acidity when kept fully saturated. The soils low in organic matter showed the greatest acidity when kept half-saturated.

(6). When the moist samples of soil taken at the close of the experiment were air-dried, the fully-saturated samples showed loss of acidity. The half- and fourth-saturated samples showed both gains and losses in acidity when air-dried.

(7) The potassium-nitrate extracts of the fully-saturated soils contained much larger amounts of iron than extracts of other samples. This soluble iron was in the ferrous form and was oxidized and made insoluble when the soils were dried.

(8) With the mineral soils the fully saturated soils had much greater amounts of soluble manganese than the other samples. Drying the soils did not render the manganese insoluble as it did the iron.

(9) There was less soluble aluminium in the fully saturated minera soils, but with the soils high in organic matter this was not true. There was both increase and decrease of soluble aluminium on drying the soils.

(10) Calcium, magnesium, and silica showed variations in solubility owing to different moisture conditions, but the variations were not as striking as those of iron, manganese, and aluminium.

(11) In correlating the soluble iron and aluminium with the acidity obtained from the potassium-nitrate extracts, it was apparent that the titrated acidity could not be entirely explained on this basis. Doubtless this acidity is partly due to soluble acid organic compounds.

(12) The measurable acidity of acid soils varies to a large degree under different conditions of moisture and aeration. These variations are due to chemical rather than physical changes in the soils.

(13) The extreme sensitiveness of the chemical compounds of soils and the wide variations caused by changing moisture conditions leads to the conclusion that some soil investigations should be conducted with undried samples.

(14) The soil moisture of acid soils is acid in reaction as shown by hydrogen-ion determinations. As the cell sap is also acid it is not necessary to consider that calcium is first changed into the form of bicarbonate before it can aid in nitrification or be assimilated by plants.

(15) A condition of acidity is produced in humid soils due to the leaching of the strong basic elements in the drainage water, by the removal of bases in crops, by the decay of carbonaceous and nitrogenous substances, and by the hydrolysis of mineral compounds and organic matter.

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DETERMINING THE ABSOLUTE SALT CONTENT OF SOILS BY MEANS OF THE FREEZING-POINT METHOD

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When the idea was first conceived of using the freezing-point method (*3*)[1] for measuring the concentration of the soil solution directly in the soil mass, the first problem which was desired to investigate was the absolute salt content of soils. It was thought that if the method proved successful it would afford a unique and excellent means of determining the absolute salt content of soils and thus yield very important and fundamental data on the comparative absolute salt content of soils. When it was discovered, however, that the soils cause water to become unfree and that this unfree water influenced greatly the concentration of the soil solution (*2*), then a direct comparison of the absolute salt content of soils had to be abandoned, and the investigation was confined to determining the actual concentration of the soil solution of a soil at any given moisture content. In this case it did not matter if the unfree water influenced the concentration as long as it was the actual concentration which existed in the soil solution.

It was realized, however, at the very beginning that for comparative studies the error caused by the unfree water was greatly minimized when a high moisture content was employed. For making a comparison, therefore, of the effect of application of salts upon the concentration of the soil solution (*3, 4*) for determining the lime requirement of soils (*1*), and for measuring the velocity of the reaction between soils and chemical agents (*5*), high moisture content or an excess of water or solution were employed. In studying the diffusion of salts in soils McCool and Wheeting (*6*) also employed a high moisture content in making freezing-point determinations.

On account of the rather small concentration of the soil solution of natural-normal soils at very high moisture content, however, no attempt was made at the beginning to make a direct comparison of the absolute salt content of soils. It was feared that on account of this rather small concentration and on account of the influence of the unfree water, the results would not be dependable, and possibly misleading. If the concentration of the soil solution were rather high at the high moisture content, any small errors that might enter in the determination would probably not have been serious.

[1] Reference is made by number (italic) to "Literature cited," p. 336.

In conducting an investigation during the past year and a half to study the rate and extent of solubility of soils by means of the freezing-point method, it was noticed that when different classes of soil were washed until their soluble salt content was greatly reduced, their lowering of the freezing point was practically identical. For instance, the freezing-point lowering of heavy sandy loams, loams, clay loam, and clays would be about 0.007° C. and that of sands and light sandy loams about 0.005° C.

This identical depression of the different classes of soil suggested at once the idea that at a comparatively high moisture content the influence of the unfree water on the concentration of the soil solution was practically negligible, if not entirely absent. This idea led immediately to the belief that at a high moisture content or in excess of water the freezing-point method could be used to determine the absolute salt content of all normal soils with a high degree of accuracy, and thus afford a comparison of their relative absolute salt content. This belief has been amply confirmed.

There appeared one factor, however, which was thought might prevent the realization of this method, and that is the effect of air-drying upon the water soluble material of soils. It has generally been believed that air-drying causes an increase in the quantity of the water soluble material of soils. It was thought, therefore, that if air-drying did exert this influence to any appreciable extent then the method could not be used, at least very conveniently, as the procedure required the soils to be dry. The recent work of Hoagland (7), however, made it very probable that air-drying has practically no effect upon the concentration of the soil solution.

Since it was discovered that at a high moisture content the concentration of the soil solution could be determined by means of the freezing-point method with a very high degree of accuracy, it was decided to investigate the influence of air-drying upon the water soluble material of soils. The problem was investigated as follows: Samples of different classes of soil were taken from the field and washed with distilled water until practically all their free soluble salts were eliminated. As a rule, a 100-gm. sample was washed, by the percolation process, with 500 cc. of water. A portion of the washed sample was placed in the freezing tube and its freezing-point depression determined. The remaining portion was placed in the room or in the sun and allowed to dry. When thoroughly dried, 15 gm. of it were weighed out and poured in the freezing tube containing 10 cc. of distilled water and its freezing point lowering determined as before. Care was always taken to have the moisture content in both cases about the same. The study involved a large number of soils representing many types and all classes. In Table I are presented the results from a few representative soils employed.

TABLE I.—*Effect of air-drying upon the freezing-point lowering of soils*

Soils.	Natural soils before washing.	Natural soils immediately after washing.	Natural soils after washing and air-drying.
	°C.	°C.	°C.
California Okley fine sandy loam	0.027	0.006	0.006
California Yolo fine sandy loam	.020	.006	.006
California Hanford fine sandy loam	.033	.007	.007
Michigan sandy loam	.035	.005	.006
Michigan silt loam	.031	.010	.010
Wisconsin Carrington silt loam	.038	.009	.010
Wisconsin Miami silt loam	.030	.010	.011
Michigan heavy silt loam	.040	.010	.011
Kentucky clay loam	.040	.010	.011
Michigan clay loam	.040	.010	.010
California Ramona clay loam	.041	.010	.010
Michigan heavy clay loam	.048	.010	.012
Wisconsin Superior clay	.031	.010	.010
Minnesota Superior clay	.035	.010	.010
Texas Crawford clay	.040	.010	.011

An examination of the data in Table I shows at once that the freezing-point depression of the soils did not increase by the process of air-drying. It will be seen that the depression is practically the same after drying as before drying, the difference is only about 0.001, which is within the experimental error. The conclusion is inevitable, therefore, that air-drying, at least once, does not increase the quantity of the soluble material of soils.

From the above results it is also seen that the rate of solubility of soils is very slow, or that saturation is not attained very rapidly as has been claimed (*6, p. 55-56*). It took at least three hours and in some cases 24 hours for the soils to dry, and yet hardly any material went into solution during this time. These results are overwhelmingly substantiated by the very extensive investigations, to be reported later, on the rate and extent of the solubility of soils, in which it is shown that the depression of soils at optimum moisture content increases only about 0.003° C. during the first 10 days, or from 0.010° to 0.013°, and that this depression increases to about 0.025° at the end of 30 days, and to about 0.040° at the end of 60 days.

As a result of the foregoing facts it is firmly believed, therefore, that the freezing-point method can be used to determine the absolute salt content of soils at high moisture content with a very great degree of accuracy.

The procedure which has been adopted for making a comparative study of the absolute salt content of all kinds of soils is as follows: The soils are allowed to air-dry if freshly taken from the field. Then a 15-gm. sample of soil is taken and poured into the freezing tube containing 10 cc. of distilled water. The soil is stirred, usually by shaking, allowed to stand for a few minutes, and its freezing-point depression determined.

For accomplishing the latter the tube is placed directly in the ice mixture having a temperature of about −2.5° C., and the soil is stirred constantly with the Beckmann thermometer until the temperature falls to about 1 degree above the zero point of the thermometer. Then it is allowed to remain undisturbed until the temperature falls to about 0.5 degree below the zero point, when the soil is again stirred with the thermometer in order to cause solidification to take place. As soon as solidification begins, the tube is at once taken out of the ice mixture and placed in the air jacket in the same bath. The soil is gently stirred and the thermometer gently tapped and the freezing point read by means of a lens. By this procedure it takes only about 10 minutes to make a freezing-point determination.

The proportion of 10 cc. of water to 15 gm. of air-dry soil has been found to be the best, as it gives a sufficient amount of excess water to practically all classes of soil, except peat, muck, and some soils containing an exceedingly high content of organic matter. Where a comparison of the salt content of all kinds of soil (with a few exceptions) is desired, therefore, the above proportion is the best. On the other hand, where a comparison of the salt content of light soils is desired, the proportion of 10 cc. of water and 20 gm. of soil is more advisable. The best ratio is that which gives a sufficient amount of excess water and at the same time a comparatively high concentration. In the case of alkali soils a ratio of one of soil to five of water may be used.

The salt content of soils can be expressed both in degrees of depression and in parts per million of solution. The latter can be easily and conveniently calculated by following the formula that a depression of 0.004° C. is equivalent to 100 p. p. m. of solution (*4*).

In determining the salt content of natural soils from the field the following factors should always be taken into consideration in interpreting the results: (1) Season of the year in which the soil is collected; (2) amount of rainfall and length of period elapsed after the rainfall before sample is collected; (3) temperature and rate of evaporation; (4) cultural conditions of the field, whether cropped or uncropped; and (5) depth of collecting sample, etc. All these factors play a very great part, if not the controlling rôle, in the amount of salts found in soils. Thus, for instance, in the early spring, when the soils are thoroughly washed by the melted snow and the spring rains and when the rate of solubility and nitrification are slow on account of the low temperature, the salt content of all soils, including the richest soils, is exceedingly small, amounting, as a rule, to a depression of only about 0.010° C., or 250 p. p. m., when the ratio of water to soil is about 1 to 0.7. In summer time after a long drouth the salt content of bare soils at the surface is quite high, amounting in some soils to a depression of 0.200° C., or 4,878 p. p. m. of solution, when the ratio of water to soils is 1 to 0.7. Immediately after a heavy and prolonged rain, however, all these salts are

leached away, at least from the upper layers, and the salt content falls again to the depression of about 0.010° C., or 250 p. p. m. at the surface. In the cropped soils the salt content is, as a rule, quite low, amounting to a depression of only about 0.010° C., or 250 p. p. m. at the various depths, as compared to a depression of about 0.200° C., or 4,878 p. p. m. in adjacent bare soil at the surface. Again, after a long drouth the salt content of nearly all bare soils varies considerably at different depths. The variation may range from about 5,000 p. p. m. at the surface to about 250 p. p. m. at the third inch. All these facts find ample confirmation in the data presented in Table II, which shows the salt content of a certain number of soils under the conditions described above. The salt content is expressed both in freezing-point depression and parts per million of solution.

TABLE II.—*Salt content and freezing-point lowering of soils at different seasons, at different periods, under different cultural conditions, and at different depths*

Description of soils.	May 10; after a rainfall.		May 18; 8 days after a rainfall.		June 12; 12days after a rainfall.		July 1; after a rainfall.		July 10; 10 days after a rainfall.	
	°C.	P. p. m.	°C.	P. p. m.	°C.	P. p. m.	°C.	P. p. m.	°C.	P. p. m.
(1) Sand, bare, 2 inches	0. 010	250	0. 015	375	0. 016	400	0. 010	250	0. 018	450
(2) Sandy loam, bare, 2 inches	. 011	280	. 017	425	. 033	825	. 010	250	. 035	875
(3) Sandy loam, bare, 2 inches	. 011	280	. 017	425	. 038	950	. 012	300	. 050	1, 250
Same as No. 3, under wheat, 2 inches	. 009	225	. 011	280	. 010	250	. 012	300	. 012	300
(4) Sandy loam, bare, 2 inches	. 011	280	. 016	400	. 028	750	. 012	300	. 033	825
Same as No. 4, under hay, 2 inches	. 010	250	. 012	300	. 013	317	. 011	280	. 013	317
(5) Heavy loam, bare, 2 inches	. 012	300	. 018	450	. 028	700	. 013	317	. 045	1, 175
(6) Heavy clay loam, bare, 2inches	. 012	300	. 019	475	. 048	1, 200	. 013	317	. 065	1, 625
Same as No. 6, bare, at surface	. 012	300	. 028	700	. 067	1, 700	. 012	300	. 130	3, 250
Same as No. 6, under oats, 2 inches	. 011	280	. 013	317	. 015	375	. 011	280	. 012	300
(7) Clay, bare, 2 inches	. 011	280	. 015	375	. 018	450	. 011	280	. 025	675
Same as No. 7, under sod, 2 inches	. 010	240	. 012	300	. 013	317	. 010	250	. 013	317
(8) Clay, bare, 2 inches	. 011	280	. 015	375	. 020	500	. 011	280	. 027	675
Same as No. 8, bare, at surface	. 011	280	. 022	550	. 030	750	. 011	280	. 035	875

It is evident, then, that the salt content of soils is controlled by many external factors, and that when these factors are not taken into consideration in the collection of the soil samples and in the interpretation of the results, very erroneous conclusions will inevitably be drawn as to the comparative salt content of soils. Yet it is surprising how many investigators in the past have overlooked or ignored these factors and

and have collected samples of soils irrespective of season, rainfall, condition of soil, date, and uniformity of sampling, etc., and then tried to compare the absolute salt content of these soils.

By being able now to determine the absolute salt content of soils, the value and usefulness of the freezing-point method are increased tremendously. This method is now able to accomplish at least two very important and fundamental things: First, to determine the actual or real concentration of the soil solution as it actually exists in the soil from a very low to any maximum moisture content, and second, to determine the absolute salt content of soils at a high moisture content. It seems to be the best, most unique, and most accurate method we have to-day for accomplishing these purposes.

SUMMARY

In the present paper the freezing-point method is presented as an excellent means for determining the absolute salt content of soils with a very high degree of accuracy. Formerly the method could determine only the actual concentration of the soil solution as it existed in the soil at different moisture contents, but now the method has been developed to measure also the absolute salt content of soils and thus afford an accurate comparison of the absolute salt content of soils.

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SWEET-POTATO STORAGE-ROTS

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INTRODUCTION

The production of sweet potatoes (*Ipomoea batatas*) in the United States in 1917 was estimated to be a little over 87,000,000 bushels, valued at more than $95,000,000 (Monthly Crop Report, December, 1917). Of this quantity probably about 50,000,000 to 55,000,000 bushels were placed in storage either to be put on the market or to be used for home consumption throughout the winter months. While no accurate data can be given, it is estimated that only about 33,000,000 to 38,000,000 bushels of the stored crop were actually consumed; the remainder were destroyed by storage-rot organisms before they reached the market. Based on the estimated average price of $1.10 per bushel for December, 1917, the loss would be approximately $18,000,000.

This article deals with the rots caused by 17 fungi responsible for the loss of sweet potatoes in storage. Several of these organisms are of minor importance, the largest percentage of decay being caused by only a few very destructive forms. In view of the fact that we are dealing with the underground parts of the plant and the method of storing the roots, it is only natural that a large number of fungi could be isolated. Many of these proved to be only saprophytes; others were able to cause decay only under especially favorable conditions.

The first phase of the problem consisted in making a collection of the organisms found in the decayed roots or in the lesions of stored potatoes. Accordingly about 40 different fungi and several species of bacteria were isolated. Twenty-five of these fungi were tested out to determine whether they were secondary invaders, saprophytes, or weak parasites capable of causing decay under certain conditions. Of this number 17 were found to exhibit varying degrees of parasitism, the remaining 8 being regarded as secondary invaders or as saprophytes. Some of the forms most frequently isolated, in fact, almost always present, were found to be weak parasites or strictly saprophytes. It was soon discovered that two and often more forms could be isolated from the same rotted root. Which of these was the primary cause of the decay could only be determined by inoculation with pure cultures. The isolation of

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a single fungus in pure culture was no guaranty that it caused the rot, since it was found that the causal fungus often died out early and a secondary form, the only one isolated, took its place. This was especially true of decay caused by *Rhizopus nigricans*. Again a fungus was isolated in pure culture from freshly decayed spots or from rotted ends of the potato, and persistently failed to cause decay when inoculated into healthy roots. *Fusarium oxysporum* is a notable example. This organism has been isolated literally hundreds of times, and the writers believe that it is one of the most common endrot organisms, but they have failed to prove it responsible for the decay.

Some of the organisms which the writers are classing as storage-rot fungi are rarely isolated, and under natural conditions evidently are responsible for very little loss. However, they will produce decay when inoculated into healthy potatoes, held under suitable conditions of temperature and humidity, and protected from the competition of other fungi. They are mostly slow-growing forms and it may be possible that they are crowded out by the more rapidly growing saprophytes. It is these organisms the writers propose to term the "minor rot-poducing fungi."

The study of sweet-potato storage-rots was begun in 1912. It has been pushed continuously ever since as a major problem of the sweet-potato disease work, except for a few months in the summer when press of field work, or the want of potatoes, made it imperative to suspend the work temporarily.[1]

A mass of data has accumulated during the period of five years that these investigations have been under way. While it can not be said that the problem is finished it is felt that the results so far obtained justify publication at this time.

METHOD OF TESTING PARASITISM

Contrary to the earlier predictions of the writers, the parasitism of most of the organisms was difficult to demonstrate. Organisms frequently isolated and always from the same type of decay, and of which there could be little question of their causal nature, would not produce rot by the ordinary simple moist-chamber methods nor consistently under storage-house conditions. We were therefore forced to turn our attention to an inquiry into the conditions required to permit decay. It has been notable throughout the entire course of the work that those organisms which appeared unquestionably responsible for decay under natural conditions would not cause rots when inserted into the host unless special methods of technic were employed.

[1] Mrs. Ethel Field Tillotson was associated in this work until the time of her retirement in 1915. She is entitled to much credit for painstaking work in the preliminary study of these diseases and especially for the development of technic for studying some of the rots. During the winter of 1915 and 1916 Mr. C. W. Carpenter entered upon the work, but after a few months left to engage in pathological work elsewhere.

The initial experiments consisted mainly in smearing spores from pure cultures, or hyphæ of the sterile fungi, on unwounded or on cut surfaces of healthy potatoes. They were then placed in moist chambers with wet filter paper in the bottom and under the lid and kept on a table in the laboratory. Almost without exception the potatoes remained sound. These preliminary experiments were repeated, but differed in that each potato after inoculation was wrapped in moistened filter paper and then in oiled paper. The results again were negative, a few being infected with softrot but no more than in the controls.

A considerable number of tests were made with the different organisms by inoculating sterile cut blocks of raw sweet potatoes in test tubes with a little water added. While the results so obtained were not entirely satisfactory, they suggested the more promising rot-producing organisms. Some of these inoculated tubes were placed in the various chambers of the Altmann thermostat, the temperature of which ranged from nearly zero to 40° C. It was shown thereby that temperature was one of the controlling factors, and other experiments showed that humidity was equally important with most organisms. The importance of humidity suggested at this stage of the work that although the potatoes were in a nearly saturated atmosphere in a moist chamber the spores were not suspended in sufficient moisture to permit germination. It is a fact easily recognized that moisture may condense on a damp chamber and yet the potatoes remain dry. Evidently potatoes, as already reported (*18*),[1] absorb the moisture from the surface, leaving the spores without sufficient water in which to germinate. This difficulty was somewhat overcome by pouring sterile water in wells about 1 cm. deep and about 1 cm. in diameter made in the potato with a cork borer. Spores were suspended in the water and the top sealed over with a cover slip set into vaseline. It was hoped by this means to retain the moisture long enough to allow the spores to germinate. This practice yielded better results than any previously tried, but was by no means satisfactory or consistent, the water being often absorbed before the spores had time to germinate. In the belief that previously germinating the spores might be a solution of the difficulty, a decoction from sweet-potato roots was made as follows: 500 gm. of sweet potatoes were finely cut into 1,000 cc. of distilled water, the whole steamed for one hour, and then filtered through gauze. If needed for immediate use, 2 cc. of this solution were put into test tubes and autoclaved for 15 minutes at 11 pounds' pressure. Generally a considerable quantity of stock solution was prepared for future use. This was put into large flasks and steamed for 20 minutes on three consecutive days. When needed, 2 cc. of this stock solution were transferred to test tubes, which after autoclaving as above were inoculated with the spores of the fungus under investigation. After about 24 hours, and rarely

[1] Reference is made by number (italic) to "Literature cited," p. 366–368.

over 48 hours, this decoction together with the mycelium, which often formed a pellicle, was poured into a well in the potato made as described above and sealed over with a cover slip set in vaseline.

The inoculated potatoes were then put into moist chambers with wet filter paper in the bottom. Inoculations by this method were usually successful, except for organisms that have been classed as strictly saprophytes. By this method sufficient moisture and nutrient substances were supplied to give the fungus a start. The method was later modified somewhat to facilitate manipulation. Instead of covering the well with a cover slip, the hole was plugged with cotton. If tightly plugged, enough of the decoction was retained to serve the desired purpose even though the specimen was turned with the well to one side or downward. Coupled with the proper temperature conditions, such extreme methods usually gave positive results with organisms that otherwise consistently gave negative ones. It may be argued that where such extreme measures are required the organism should not be classed as a rot producer. The only answer to this is that in our hands they are rot producers under artificial conditions only when subjected to this test and most of the organisms studied require such a method. This applies to some of the most common forms consistently isolated from a rot of a definite type, and of which there can be little question of their causal relation.

SOFTROT AND RINGROT

There are two storage-rots of sweet potatoes caused by *Rhizopus nigricans* Ehrbg.: Softrot and ringrot. The former has long been known as the softrot of sweet potatoes in storage. Halsted (*12*), who, we believe, was the first to recognize it as such carefully described it in 1890. In 1892 a rot of quince (*13*) was attributed by him to the same fungus. Since that time there have been other observations and experiments which show that *R. nigricans* is responsible for rots of fruits and vegetables under suitable conditions. Behrens (*3, p. 515–516*), Wormald (*41*), and Hanzawa (*16*) have all found it causing a rot of tomatoes. In 1908 Morse (*28*) attributes a rot of fruit to it, and Orton (*29*) a year later found the same fungus associated with the "leak" of Irish potatoes in the San Joaquin Valley of California. Edgerton (*10*) attributes a heavy loss of figs to the same fungus. In 1916 Stevens and Peterson (*32*), in a study of strawberry fungi, mention *R. nigricans* as the cause of considerable loss to the berries at their destination. A more extensive study of this same trouble was later made by Stevens and Wilcox (*33*), who conclusively proved the parasitic nature of the fungus and studied the factors which contribute to its destructiveness. While this brief survey of the literature shows *R. nigricans* to be a relatively common rot-producing organism of various hosts, it is best known as the cause of softrot of the sweet potato, to which it probably causes more monetary loss than to all the other crops combined. Compared with other organ-

isms causing decay of sweet potatoes in storage, it is certainly the most destructive and probably causes more loss than all the others combined. In fact, there could be no doubt in the mind of anyone who had carefully observed and studied softrot under natural conditions that *R. nigricans* was responsible for it. However, to determine the conditions required to bring about decay and consistently to produce it artificially is quite another matter.

Ringrot, now known to be caused by *R. nigricans*, was originally thought by Halsted (*14*) to be due to *Nectria ipomoeae* Hals., but was later shown (*35*) to be caused by *R. nigricans*.

CHARACTERISTIC SYMPTOMS

The so-called softrot begins at one of the ends of the potato, occasionally in the middle, and progresses rapidly through the healthy tissue. Only four to six days are required to complete the destruction of the entire potato at room temperature. This time may be shortened by higher temperatures, and correspondingly lengthened by lower temperatures. Humidity seems essential only in so far as it contributes to the initial infection of the potato. Experiments have shown that decay, after once having started, will continue, though slightly retarded, even in an atmosphere almost entirely free of moisture, the fungus apparently being able to obtain the needed moisture from the host. These results are in strict accord with those obtained with strawberries by Stevens and Wilcox (*33*). The potatoes are at first rendered very soft and stringy, water often dripping out of the potato when broken open. It has a characteristic mild yeast odor at first, followed by a wild-rose to rose-geranium odor later. At the outset the color of the tissue is not changed, but later it turns a cinnamon to chocolate-brown. If the epidermis of a decayed potato is ruptured, the sporangiophores and sporangia develop in great numbers in the air (Pl. 21, A). On the escape of moisture the potato dries up and finally becomes dry and mummified. Observed in this stage it is often classed as a dryrot.

Usually softrot sets in soon after the potatoes are put in storage and continues more or less throughout the entire storage period, depending largely upon weather conditions and the management of the storage house. It is believed that the rot does not depend to any extent upon the amount of sugars and starch present; evidence in support of this theory will be presented later. Softrot is largely a storage trouble, though it is occasionally found in the field at digging time in wet soils, especially those containing a considerable amount of organic matter.

Ringrot differs from softrot only in that the infection occurs at one or more places between the two ends. It progresses around the potato forming a ring or collar, by the drying out and subsequent shrinking away of the rotted tissue, as shown by Plate 21, B. The extent of the rot varies, being in some cases 1 or 2 inches in width, and may extend

½ inch in depth, or entirely through the potato. It may dry up after completing the ring, or it may advance toward the two ends and finally complete the destruction of the entire potato. As many as three such rings have been seen on a single potato, both in storage and in the field. Taubenhaus claims that it is only a storage-rot, but the writers have found it more prevalent in the field than softrot.

DISSEMINATION

In view of the general prevalence and wide distribution of the fungus, it is doubtful whether dissemination of *R. nigricans* is ever necessary to insure infection. Although the storage house may have been thoroughly disinfected, it is likely that the spores are carried in on the potatoes, where they remain dormant until conditions are favorable for their germination and infection of the host. Such an environment is soon provided after the sweet potatoes reach the storage house. During the sweating and curing period, when the temperature is high, a certain number of potatoes are softrotted. Upon the rupture of the epidermis sporangia form in great abundance on the surface. The spores are then easily distributed by insects which frequent such rotted potatoes, by settling in the bins, and possibly by air currents. A certain amount of spore dissemination may also be brought about by workmen preparing the potatoes for market. That the disease is communicable is evident from the fact that often a number of potatoes in contact with each other have rotted at about the same time. The rotting of a number of potatoes in contact is much more common in the center of the bin, where ventilation is poor. Individual rotted potatoes on the top of the bins and elsewhere, however, are frequently found. Experiments have shown that the hyphæ of *R. nigricans* die relatively soon. The spores, on the other hand, remain viable for several months.

INOCULATION EXPERIMENTS

It has already been stated that there could be little doubt in the mind of anyone who had studied softrot and ringrot under natural conditions that *Rhizopus nigricans* had caused it. Taubenhaus (*35*) and Taubenhaus and Manns (*37*) published results in which they claim to have brought about the complete decay of the potatoes and the formation of mature sporangia on the surface in 15 hours by smearing the spores dry on the surface of the potatoes in a moist chamber. Their results are not supported by those of the writers. With several hundreds of transfers of *R. nigricans* on various media and on sweet potato decoction, the best medium so far tried, 24 to 48 hours were required to produce sporangia at room temperature. Ames (*1*) who investigated the temperature relations of *R. nigricans* among other storage-rot organisms, found that the shortest time in which the spores could be germinated was 5½

hours at a temperature of 38° to 41° C. No germination occurred at 42°. In general, she found that the length of time required to germinate the spores increased as the temperature decreased. At 25° and 20°, temperatures more nearly approximating room temperature, 13 and 16 hours, respectively, were required to germinate the spores.

In a large number of experiments the writers have been unable to verify the results of Taubenhaus (*35*) and Taubenhaus and Manns (*37*) by the method they used. Neither have satisfactory results been obtained by inserting spores and hyphæ deep into healthy tissue and confining the potatoes in a damp chamber. Potatoes inoculated by wounding and wrapped in wet filter paper and then in oiled paper to retain the moisture would not give positive results. Potatoes cut in two lengthwise and the spores and hyphæ confined between the two halves would not rot any more than the controls, even when wrapped in moist filter paper and oiled paper and subjected to the environment of a moist chamber. Spores suspended in water in a well made in the potato and sealed over by a cover slip would not give consistent results. Chilling the potatoes or bruising before inoculating likewise failed to give anything like conclusive results. Experiments of this type in large numbers have been made on potatoes of all sizes taken from storage houses at different times during the storage period and always with negative results. The writers have even failed to get results by cutting off the rotted end from a softrotted potato and inserting bits of the diseased tissue from the rotted part into the sound portion.

Similar results have been obtained independently by Dr. Heinrich Hasselbring, of the Bureau of Plant Industry, who has permitted the writers to use his results. He made literally hundreds of inoculations from pure cultures of *Rhizopus nigricans* into jagged wounds made with a scalpel. The potatoes were then kept in moist chambers or under bell glasses at room temperature. Now and then a potato rotted, but not enough to justify the conclusion that consistent infection had been obtained.

Not until the writers had developed the "well method" described on page 339 could they rot the potatoes at will. A 24-hour growth of *Rhizopus nigricans* in sweet-potato decoction poured into a "well" and protected against too rapid drying out would almost always result in rotting the potato. By this method rot has been produced in large as well as small potatoes and in sprouted as easily as unsprouted. In view of the fact that stored sweet potatoes could be rotted with equal ease at any time of the storage period by this method, it would seem that the sugar content of the potatoes, if contributory to infection, certainly was not a controlling factor. Hasselbring and Hawkins (*24*), in an exhaustive series of experiments, have shown that at digging time the starch content is high and the sugar content of the sweet potato is low. From the beginning of the storage period the percentage of starch gradually

decreases and the sugar content increases up until about March, when there is a slight reversal of the process. They have further shown that about March or April, when the sugar content is highest, the water content is not higher, but actually lower. There is evidently enough sugar during any of this time to supply the needs of the fungus. In fact, sweet potatoes growing in the field have been successfully infected with *R. nigricans*, and it is a common thing to see naturally infected potatoes in the field at digging time and in the hotbed in the spring. The writers have been able to infect freshly dug potatoes at will, as well as potatoes in storage as late as June.

BLACKROT

We owe our first knowledge of blackrot, caused by the fungus *Sphaeronema fimbriatum* (E. and H.) Sacc., to Halsted (*12*), who found it causing much damage to the sweet-potato crop in New Jersey. In 1890 he published a very good account of the disease, which he attributed to *Ceratocystis fimbriata* E. and H. This name was later changed to *Sphaeronema fimbriatum* by Saccardo (*31*). The following year Halsted and Fairchild (*15*) published the results of an excellent morphological study of the blackrot organism. In fact our knowledge of this fungus has been little advanced since the publication of their work. The sclerotial bodies which were thought by them to be a stage in the development of the blackrot fungus were later shown (*34*) to be a separate organism. On the whole, however, the blackrot fungus was the best known, and its life history and morphological characters better understood, than any of the other organisms causing sweet-potato diseases. Chester (*6*), contemporaneously with Halsted, came to the conclusion that the causal fungus was carried over in the soil. That blackrot is important as a field disease, a storage-rot, or both, and is widely distributed may be judged from the writings of Price (*30*), Duggar (*9*), Townsend (*39*), Wilcox (*40*), Carver (*5*), Barre (*2*), McClintock (*26*), and others, all of whom mention it as a field disease or in connection with storage-rot. It therefore seems imperative to discuss briefly this disease as it affects the plants in order to show how it becomes so destructive in the storage house.

If blackrotted potatoes are bedded, the slips produced therefrom will almost invariably have blackrot or "blackshank," as the disease is sometimes called. It is characterized by blackrotted areas of varying extent on the underground part of the slip. Plate 22, A, is reproduced from a photograph of a typical hotbed infection of a young plant, and Plate 22, B, shows a bedded potato the slips of which have been killed by the blackrot organism. The fungus not only reaches the hotbed by being carried there on the seed potatoes, but it will live over in the soil of the old hotbed or in other soils where infected plants have been grown. Diseased plants, if set in the field, serve as a source of infection to the new crop. It is

these potatoes, infected in the field, which carry the disease to the storage house. Usually when the potatoes are put in the storage house they show little or no evidence of blackrot. The potatoes with visible blackrot spots are few, and such are generally thrown out. On the other hand, those with infections too small to be seen pass along into the storage house, where they continue their development. In the course of a few weeks the spots attain a diameter of an inch or more, and the fungus, under suitable conditions, has fruited abundantly. The spores are scattered about the house on the bodies of insects, by the settling of the potatoes in the bins, and probably by currents of air, and by other means, such as picking the potatoes over and preparing them for the market. In some houses a large percentage of the potatoes have blackrot, though at the time they were put in storage they showed no evidence of it. Plate 23, A, shows a potato infected with blackrot and Plate 23, B, the same potato two months later. This potato was kept in an ice box at a temperature of 10° to 13° C. During the two-month period the spots had developed so as to envelop nearly the entire potato.

That blackrot is transmitted through the soil was demonstrated by bedding a large number of healthy potatoes in soil infested with blackrot. The potatoes used in the experiment were carefully examined for soundness, and then disinfected in mercuric chlorid (1:1,000) for 10 minutes. Potatoes from the same lot, bedded in disease-free soil in the same hotbed, were used as controls. The slips from blackrotted soil were set alongside the control plants in a field where blackrot was not known to occur. Only a few of the slips when set out showed blackrot infection. About 25 bushels of potatoes were produced from these slips. When the potatoes were dug, a few had visible blackrot spots on them and were discarded. The apparently sound potatoes (25 bushels) were mixed with sound potatoes in a 100-bushel bin. When they were removed in the spring, most of the 100 bushels in the one bin had to be thrown out, and a fair percentage from bins on either side. The loss in the adjoining bins was greatest on the side next to the bin containing the blackrotted potatoes. The disease had not been communicated to any extent to other bins in the same house. The potatoes from the control plants stored in another part of the house all remained sound. Further proof that it is disseminated in storage was shown by an experiment in which blackrotted potatoes and healthy potatoes were mixed in a bushel basket and stored. When the potatoes were removed in the spring, all but one of the healthy potatoes were infected with blackrot.

DESCRIPTION OF BLACKROT

Blackrot is characterized by the formation of more or less circular, somewhat sunken, black spots on the surface of the potato. Infection takes place readily through wounds and through the dead rootlets. If

the potato is cut open with a knife and spores smeared on the wound, the organism will cover the entire cut surface in a short time if sufficient moisture is provided. Likewise, if unwounded potatoes in a moist chamber are sprayed with the spores, numerous infections will result, a small rootlet usually being the center of the spot (Pl. 24, A). It is likely, also, that under natural conditions infection takes place in a similar manner with or without wounding. Pycnidia usually develop in the center of the infected area at the point where the fungus entered, or at any place where the epidermis may be ruptured. Under natural conditions there are usually only a few spots on a potato, although in exceptional cases there may be many. Plate 24, B, shows a spot on a potato after several weeks in storage. If kept in storage for several months, the entire potato may be involved and rendered useless for food.

In most infections the fungus penetrates only to the vascular ring, though it has often been isolated in pure culture from the center of the root (Pl. 24, C). Taubenhaus (*34*) claims some resistance for the small roots, but the writers have been able to infect them, and even the rootlets, as easily as the large ones.

Blackrot specimens have been collected or received from every State in the Union where sweet potatoes are grown. It can safely be said that the disease is as widely distributed as the crop itself.

SUSCEPTIBLE VARIETIES

Growers of sweet potatoes are always interested in knowing whether there are any varieties resistant to this or any other disease with which they are concerned. Although no inoculations were made, records have been kept for several years of the varieties infected under natural conditions. These records were obtained either from varieties identified by the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry, or from varieties obtained from that office and subjected to natural infection of blackrot. To this have been added data collected on visits to the various sweet-potato growing sections of the country. The following varieties were found susceptible: Southern Queen, White Yam, Big Stem Jersey, Yellow Jersey, Red Bermuda, Red Brazilian, Florida, White Gilke hybrid, Vineless Pumpkin Yam, Pumpkin Yam, Eclipse Sugar Yam, Porto Rico, Triumph, Yellow Yam, Early Carolina, Miles Yam, Georgia, Pierson, Key West Yam, Nancy Hall, Red Jersey, and an unnamed variety, No. 10950 (Horticultural and Pomological Investigations number). In all, 21 varieties are known to be susceptible to blackrot. As this list includes most of the best known and most widely cultivated varieties of sweet potatoes, it is doubtful whether any of the varieties are resistant to blackrot.

JAVA BLACKROT

Java blackrot, a disease caused by *Diplodia tubericola* (E. and E.) Taub., is probably as widely distributed as the sweet-potato crop itself, and the total loss from this disease is large. It has been collected from every part of the United States, and specimens have been sent to the writers from Cuba, Isle of Pines, Philippine Islands, Japan, Porto Rico, South America, and elsewhere. It can be found in practically every sweet-potato storage house and also in the banks. This disease causes a greater loss in the Tropics and the southern part of the United States than in the northern sweet-potato belt.

Java blackrot was first reported on sweet potatoes in 1896 by Clendenin (*7*). The specimens on which she observed the disease were sent from Java to the Louisiana Experiment Station in 1894. From what we know at the present time, however, it is likely that this disease had been common in this country long before then, probably ever since sweet potatoes have been cultivated. The fact that it was first reported on sweet potatoes imported from Java is no evidence that it was introduced at that time. We now know that species of Diplodia occurring on various hosts will also infect sweet potatoes. Investigations have shown (*36*) that *D. gossypina* Cke., *D. natalensis* Ev., and *Lasiodiplodia nigra* Appel and Laub., will all cause a rot of sweet potatoes similar to the rot caused by *D. tubericola*. Furthermore, it has been shown by the senior author (*18*) that *D. tubericola* from dasheen (*Colocasia esculenta*), *D. gossypina*, *D. maclurae* Speg. and *Diplodia* sp. from mango (*Mangifera indica*) will produce a rot of sweet potatoes identical with the rot produced by *D. tubericola* isolated from sweet potatoes. Further evidence of the cosmopolitan nature of these organisms has been submitted by Meier (*27*), who found that *D. tubericola* from sweet potato would cause the stem-endrot of the watermelon (*Citrullus vulgaris*).

It is therefore probable that species of Diplodia from other hosts, if inoculated into sweet potatoes, dasheens, or watermelons, would cause similar rots.

DESCRIPTION OF JAVA BLACKROT

Diplodia tubericola rots sweet potatoes very slowly. Under laboratory conditions there is little or no evidence of decay for a week or 10 days, and usually 4 to 8 weeks are required to entirely destroy a potato. The pycnidia, which ordinarily develop in great abundance, generally appear at the end of one month on the part of the potato first decayed. They are externally coal black, crowded closely together or confluent, and microscopically suggest minute domelike elevations on the surface. Unlike some of the fungi of this group, many pycnidia are completely buried, the spores escaping only after maceration or disintergation of the host. The tissue is first rendered brown in color, but later becomes coal black and hard. Concomitant with the loss of water, the root shrinks, eventu-

ally becoming mummified (Pl. 25, A). The spores may be of three types and sometimes all three may be found in the same pycnidium. In the young pycnidium they are usually hyalin and 1-celled, and occasionally this is the only type found; but usually a little later the hyalin spores turn dark, and may or may not be septate. In the old mummied potato the 2-celled dark spores predominate, but are intermixed with a few 1-celled dark and a few 1-celled hyalin spores.

INOCULATION EXPERIMENTS

The first inoculation experiments were made on January 16, 1914, when spores and hyphæ were inserted into a wound at the end of eight healthy roots, four with *Diplodia tubericola* from dasheen and four with the same organism from sweet potato. These potatoes were kept in an uncovered vessel in the laboratory. This method was followed because preliminary experiments showed that better results could be obtained by exposing the inoculated roots to the dry conditions of the laboratory room than in a moist chamber. Just why this should be has not been determined. On February 18, six of the potatoes were partially rotted and on March 12 all but two, which remained sound, were completely decayed, *D. tubericola* being recovered in pure culture from each. On May 8, eight potatoes were inoculated with *D. maclurae* and placed in an uncovered moist chamber. One of these potatoes remained sound, but the others were completely decayed by July 11.

On September 2, 1914, twelve sweet potatoes were inoculated with *Diplodia gossypina*. Six were inclosed in a damp chamber with moist filter paper in the bottom, and six were placed in an open receptacle. On October 15, in the open vessel the results were as follows: Two were completely rotted, two one-fifth rotted, and two sound. The fungus was recovered from the four decayed potatoes. In the moist chamber one was completely rotted, one one-third rotted, and four sound. From one of the rotted potatoes *D. gossypina* was isolated, and from the other an unknown fungus.

On January 5, 1915, six sweet potatoes were inoculated with *Diplodia zeae* (Schw.) Lev. All remained sound.

On January 14, 1915, ten sweet potatoes were inoculated with *Diplodia tubericola* from sweet potato. Five were immediately placed in an incubator (34°–35° C.), and five in an ice box (12.2°–13.5° C.). On February 8 two potatoes in the incubator were entirely rotted, and one remained sound. On February 23 one of the two remaining potatoes was half decayed and the other entirely decayed. There was no evidence of decay in the potatoes in the ice box on January 25, but by January 29 two had been completely rotted by *Rhizopus nigricans*. By March 31 two were partially rotted, and *D. tubericola* was isolated in pure culture. The remaining potato was completely decayed, and *Alternaria* sp. was obtained in pure culture from it. The best results were obtained at the higher temperature.

On March 1, 1915, six sweet potatoes were inoculated with *Diplodia tubericola* from dasheen and kept in an open receptacle. One potato was decayed by *Rhizopus nigricans*. By June 1 two were entirely decayed, and *D. tubericola* was isolated from them. Three remained sound.

The question has frequently been raised whether or not *Diplodia tubericola* attacked the plants in the field and was carried on the infected roots to the storage house where it further developed. A number of half-grown plants were inoculated in the field by inserting spores and hyphæ of *D. tubericola* from sweet potatoes into the stem near the hill. When the potatoes were dug, none of the plants showed any evidence of disease. The potatoes from all the inoculated plants were stored together, but none of them were decayed by *D. tubericola*. From these results and numerous field observations it is concluded that this organism does not attack the plants in the field, and consequently could not transmit the disease to the roots.

DRYROT

Diaporthe batatatis (E. and H.) Harter and Field causes what is commonly known as the dryrot of sweet potatoes. It was first reported by Halsted (*12*) in 1890, who attributed the disease to *Phoma batatae* E. and H. Later the disease was more exhaustively studied by Harter and Field (*21*), who obtained in pure culture the perfect stage of the causal organism to which the name "*Diaporthe batatatis*" was given. The imperfect stage of the fungus is the only one found on the potatoes from the storage houses or field material. They reported the occurrence of the disease in the States of North Carolina, Texas, New Jersey, Virginia, Mississippi, Alabama, and Indiana. Since then it has been collected in many other States, or diseased specimens have been received from them, so that it can be safely said to have a wide distribution. In 1917 *D. batatatis* was isolated from material from the Isle of Pines and when inoculated into healthy plants on the Potomac flats near Washington D. C., produced characteristic symptoms of the disease. This strain, while identical morphologically, is a more vigorous parasite than any isolated from material collected in the United States.

Although this organism is quite prevalent, the total loss from the disease it produces is relatively small, the loss being more in storage than in the field, though it is occasionally found on the slips in the hotbed. Like many other fungi, it will grow as a saprophyte and is for this reason found also as a secondary invader. Inoculation experiments have shown that it is quite capable of causing decay.

Diaporthe batatatis usually enters the potato from the stem end and progresses slowly downward. It grows very slowly, requiring 4 to 8 weeks to entirely destroy a potato. In this respect it resembles *D. tubericola*. Infected potatoes become much shrunken and wrinkled and finally mummified (Pl. 25, B). The surface, beneath which the tissue

is carbonaceous to coal-black, is covered with small elevations, a millimeter or so in diameter lying close together, in which the pycnidia are embedded.

FOOTROT

Footrot, a disease caused by *Plenodomus destruens* Harter, is one of the most serious diseases of the sweet-potato crop, once it has become established. It also occurs in storage and is carried over mostly on the potatoes held for seed. The results obtained by the writers confirm those of McClintock (*26*), who found that field infection, while not entirely lacking, is relatively small, the disease being carried to the field mostly on the slips. During the early part of the season the fungus grows slowly, but in July and August, when warm weather comes on, it progresses rapidly. It has been shown (*17*) that the footrot organism invades the stem of the plant near the ground and grows down into the potatoes, causing a decay beginning at the attached ends (Pl. 26, A). This decayed portion is generally slight at digging time and, therefore, easily overlooked. Occasionally it may involve an inch or more of the end of the potato, in which case it would probably be thrown out. Potatoes but slightly decayed may find their way into storage, where the development of the disease would be continued. By bedding time a considerable percentage of potatoes are thrown out in a badly decayed condition. Those slightly decayed, however, escape detection and, therefore, may be used for seed. The causal fungus may also enter through wounds, or invade surface lesions made by other fungi.

DISTRIBUTION

In 1913 (*17*) footrot was known to occur only in Virginia. By 1916 Iowa, Ohio, Missouri, and Kansas (*19*) were added to the list. In 1917 it was found in New Jersey, Maryland, and California. In New Jersey it is doing very little damage as yet, but in California and Maryland considerable loss resulted from it in 1917 and 1918, respectively. Just how long it has been present in California has not been learned. The organism isolated from material collected in California, when inoculated into healthy plants on the Potomac Flats, produced typical symptoms of the disease. In fact, it was found to be identical morphologically and parasitically with the strains isolated from material collected in Eastern States. The following varieties of sweet potatoes have been inoculated with *Plenodomus destruens* and the disease produced: Yellow Jersey, Big Stem Jersey, Pierson, Miles Yam, Early Carolina, Yellow Strasburg, Red Jersey, Red Bermuda, Extra Red Carolina, Southern Queen, Yellow Yam, Pumpkin Yam, Vineless Yam, Dooley, Triumph, Vineless Pumpkin Yam, Nancy Hall, Florida, General Grant Vineless Yam, White Yam, Red Brazilian, and Dahomey.

The footrot fungus produces a relatively slow decay, two to three weeks being required to destroy completely an average-sized potato. It produces a somewhat spongy rot and turns the tissue brown. Upon the escape of moisture it becomes dry, shrunken, hard, and finally brittle.

CHARCOAL-ROT

Charcoal-rot is a rather common type of storage-rot caused by *Sclerotium bataticola* Taub., and is distributed throughout the United States and elsewhere. It has been collected widely, and specimens of sweet potatoes have been received from Japan and other foreign countries decayed by *S. bataticola*. The writers have often isolated it from the lower part of the stem and underground parts of sweet-potato plants injured in the field by other agencies. It will grow saprophytically on most any substance, and occurs as a secondary invader both under field conditions and in the stroage house.

Sclerotium bataticola evidently was originally thought by Halsted (*12*) to be a stage in the life history of *Sphaeronema fimbriatum*, but after a more thorough study of the disease by Halsted and Fairchild (*15*) it was evident they entertained some doubt of its connection with the black-rot fungus. Later investigations (*34*), however, showed that the sclerotial form was *Sclerotium bataticola*, and was in no way connected with *Sphaeronema fimbriatum*.

Sclerotium bataticola is another of the slow-growing storage-rot fungi, requiring about 3 to 6 weeks to rot completely a potato under moist-chamber conditions at laboratory temperature. The decayed tissue first becomes a chocolate to a cinnamon-brown, followed by a dark reddish-brown color. As soon as the sclerotia begin forming, it becomes black or charcoal in appearance. Three distinct zones differing in color, therefore, may be distinguished in a potato at the same time. The black zone contains the sclerotial bodies. Adjacent to this is a dark reddish-brown area. The freshly decayed part is of a chocolate-brown color.

The potato in the early stages of decay is spongy, but on the escape of moisture it gradually becomes hard and mummified. In this stage the epidermis is darkened from the action of the fungus, but there is no other external indication that the potato is rotted. If the epidermis is broken, the black sclerotial bodies may be seen in large numbers. In a completely decayed potato these sclerotial bodies are buried among the cells throughout the potato.

INOCULATION EXPERIMENTS

Several inoculation experiments were carried out at different times, and in most cases when the well method described earlier was employed positive results were obtained. The writers found that *Sclerotium bataticola*, like some other storage-rot organisms, would not consistently

decay the potatoes when the hyphæ were inserted into wounds. Inoculated potatoes held at laboratory temperature and confined in a moist chamber would usually decay in from 3 to 6 weeks. A number of potatoes were inoculated on December 5, 1916, and 43 per cent were completely or nearly decayed by January 2, 1917. The causal organism was recovered from a potato rotted in the above experiment and used to inoculate another lot on January 22, 1917. Seventy per cent of the latter were from two-thirds to completely decayed by February 23.

SCURF

Scurf is a common field disease of sweet potatoes caused by *Monilochaetes infuscans* Hals. It is very prevalent in California, the entire South, and other sweet-potato growing sections of the country. Scurfy potatoes sell for from 25 to 50 per cent less on the market than clean ones, depending naturally on the severity of the infection. To this loss must be added those scurfy potatoes which are so badly dried and shrunken that they are not fit for food. The spotted discoloration of the skin is well known to nearly everyone (Pl. 26, B). These spots may be small and separate or they may coalesce so as to form a continuous discolored covering over the end or even over the entire potato. The discolored or infected areas are for the most part present in the field before the crop is harvested, though they may enlarge slightly in storage, and possibly a few new ones may be formed.

Although *Monilochaetes infuscans* is superficial, the hyphæ penetrating only through the cuticle (*19*), it injures the epidermis so that water escapes, thus resulting in a shrinkage of the potato. This shrinkage is slow, but after one or two months in storage a considerable percentage is unfit for the market. Naturally the shrinkage is greatest in houses where the relative humidity is low and the temperature is high. Plate 26, C, shows a badly shrunken scurfy potato. The shrinkage is generally worse at one end, usually the end that was attached to the stem. That there is more shrinkage at the attached end is not surprising when one remembers that scurf, like several other diseases of the sweet potato, is largely carried to the field on the slips. Being a surface organism, it readily grows from the slip on to the roots or is washed there by rains. That many of the infections on the attached ends results from the spores being washed down the stem on to the roots is evident from the fact that the infections for the most part are in spots.

There are no varieties of sweet potatoes immune to scurf, so far as the writers know. Data collected over a period of six years show that the following varieties of sweet potatoes are susceptible to scurf: Yellow Jersey, Red Bermuda, Japan Brown, Red Brazilian, Florida, White Gilke hybrid, Vineless Pumpkin Yam, Pumpkin Yam, Eclipse Sugar Yam, Porto Rico, Triumph, Yellow Yam, Yellow Strasburg, Early Carolina, Creola, Georgia, Miles Yam, Pierson, White Yam, Key West

Yam, Big Stem Jersey, Nancy Hall, Southern Queen, Dahomey, and several varieties with accession numbers but not yet named. McClintock (*26*) listed approximately these same varieties, stating that a number of them had little or no scurf when grown for a season in Virginia. The writers have found, however, that many of the varieties he listed as having little or no scurf were the most susceptible and worst affected in other sections of the country. There is, in the opinion of the writers, little difference in the susceptibility of the different varieties, since varieties which have shown a degree of resistance in one section of the country are not resistant in another.

MINOR STORAGE-ROTS

The following group of storage-rots are of minor importance. They are caused by fungi occasionally met with and which under the proper environment will decay sweet potatoes. That they have not been more frequently met may in part be due to the fact that they are slow-growing forms, and as such may be crowded out or overrun by the more rapidly growing saprophytes which follow them. This does not necessarily presuppose the killing out of the casual fungus, although such might well happen. It is a well-known fact that certain organisms do not live long in their own staling products. *Rhizopus nigricans*, for instance, will often die in two or three weeks in tissue previously destroyed by it. Other organisms, saprophytes perhaps, may find abundant food there and conditions otherwise congenial for their development. With these facts in mind it is easy to understand why a saprophyte instead of the causal fungus may be, and doubtless often is, isolated from decayed tissue. Furthermore, the causal fungus and a saprophyte may both be living congenially in the same decayed tissue, but when isolations are made the latter, if the more rapid grower, which it usually is, will so completely crowd out the parasite that only the saprophyte appears in the plate. Likewise the temperatures at which sweet potatoes are usually stored are the temperatures at which many of these minor rot-producing organisms do not grow well. It is at the low or high temperature, thus excluding some of the saprophytes, that some of these fungi grow. These fungi therefore have been found to cause rots and have been isolated from decayed potatoes held at a temperature lower than that at which sweet potatoes are usually stored.

With one or two exceptions none of the organisms have ever been reported to have produced decay of sweet potatoes in storage. No common names are known for these rots; therefore they will be discussed under the name of the causal organism.

MUCOR RACEMOSUS

In the course of the sweet-potato storage investigations potatoes have been exposed for varying lengths of time to temperature a little above freezing. After a few weeks at a temperature of 2.0° to 4.5° C. they were usually decayed, and in these rotted potatoes two or three fungi always predominated. One of the most common forms was *Mucor racemosus* Fes. In one experiment four sacks of about ¼ bushel each were stored for two months at 2.0° C., and all the potatoes were decayed with *M. racemosus*. A large number of potatoes have been inoculated at different times with pure cultures of *M. racemosus* and stored in the usual way in a sweet-potato storage house, but no infections resulted. However, in other experiments in which the potatoes were inoculated and then stored at a temperature of about 2° C. rot was produced and *M. racemosus* was isolated in pure culture after two or three months. In one such experiment 100 potatoes were inoculated and *M. racemosus* was recovered in pure culture from 82 per cent. The remaining 18 per cent were infected with an undetermined species of Mucor and five unknown fungi. That low temperatures are necessary was further proved by a number of experiments in which the potatoes were inoculated with pure cultures of *M. racemosus*, using the "well" method previously described, and confined in moist chambers. No decay resulted when these potatoes were exposed to the temperatures of the laboratory room or of an ice box (10°–14° C.). If, on the other hand, they were exposed to low temperatures, they were rotted readily, as shown by the following experiments.

In all these experiments the fungus was grown for 24 hours in sweet-potato decoction, and both the growth and the decoction poured into a "well," after which they were wrapped in filter paper and then in oiled paper. This served both to retain moisture and to prevent contamination. Before they were inoculated the potatoes were thoroughly washed and then disinfected with mercuric chlorid. After inoculation the potatoes were divided into several lots and placed in different chambers of the Altmann thermostat. In these and subsequent experiments only the average temperature covering the period of the experiment in the Altmann thermostat is given. The maximum and minimum varied 1 to 2 degrees above and below the average given. In one experiment only two chambers were used—viz, chamber 1 (average temperature 0.43° C.) and chamber 3 (7.2° C.). In three weeks 100 per cent of the potatoes in both chambers were from half to completely decayed. The experiment was repeated, using chambers 1 (0.67° C.), 2 (4.37° C.), 4 (8.1° C.), 5 (11.6° C.), 7 (14.7° C.), 10 (22.8° C.). In chambers 1, 2, and 4 all the potatoes rotted. The above experiment, in which 10 potatoes after inoculation were placed in chambers 2 (5.34° C.), 3 (7.81° C.), 4 (8.7° C.), 5 (11.0° C.), 7 (15.8° C.), 9 (17.5° C.), laboratory room

(23° C.), and an incubator the temperature of which averaged about 28° C., was twice repeated. In chambers 2 and 3, 70 per cent in the one series and 90 per cent in the other were decayed. The sweet potatoes at all other temperatures remained sound.

The results seem to indicate that *Mucor racemosus* produces a much slower rot than *Rhizopus nigricans*, about two to three weeks being required to complete the destruction of a sweet potato under optimum conditions. The tissue is rendered a clayish white in spots, as shown in cross section (Pl. 27, A). It is somewhat wet, but spongy to firm. When pulled apart or broken open, it pulls out in a fibrous, stringy manner. It has a distinct starchy odor.

ALTERNARIA SP.

Alternaria sp. is another fungus we have frequently isolated from sweet potatoes held at low temperatures. Owing to its prevalence under such conditions, inoculations were made by inserting spores and hyphæ into the end of some potatoes and exposing them to the temperature of chambers 3 (7.19° C.), 6 (14.4° C.), 9 (20.9° C.), 11 (26.0° C.), 16 (30.6° C.) of the Altmann thermostat. Each sweet potato was wrapped in filter paper and then in oiled paper. All the potatoes in chamber 3 were partially rotted in 19 days. In chamber 6 only a slight rot had started and at the higher temperatures all the potatoes remained sound to the close of the experiment.

Alternaria sp. produces a firm, moist rot. The tissue is first turned brown and then gradually darkens, but never becomes black (Pl. 27, B). The potato breaks easily, and the parts separate without the formation of strands so characteristic of soft rot.

PENICILLIUM SP.

The species of Penicillium which we most frequently isolated and with which all our work has been done was given to Dr. Charles Thom, of the Bureau of Chemistry, for identification. According to his determination it belongs to the *expansum* group, and is similar to a number of other strains for which there are no specific differences. It was a common inhabitant of decayed sweet potatoes and generally of those that were decayed by other fungi. Occasionally it was isolated alone in pure culture, but more often it was accompanied with one or more other organisms. Like some of the other fungi already discussed, it was most often obtained from potatoes held at low temperatures. That it may be, under a protected environment, a storage-rot producing organism at low temperatures was demonstrated by a series of experiments in which chambers 2 (4.44° C.), 5 (12.0° C.), 8 (18.7° C.), 10 (22.9° C.), 12 (26.9° C.), and 17 (32.0° C.) of the Altmann thermostat were used. In chambers 2 and 5 the potatoes were from one-fourth to two-thirds decayed in 39 days.. In

the other chambers all the potatoes remained sound. While the results show some success at low temperatures from inoculating sweet potatoes with *Penicillium* sp., this fungus, even when removed from the competition of other organisms, must for the most part be considered a saprophyte.

Penicillium sp. forms blue masses of spores on the interior and on the surface of the sweet potato (Pl. 27, C).

BOTRYTIS CINEREA

Botrytis cinerea Pers. was often isolated from sweet potatoes, as well as dasheens and other vegetables held at low temperatures. So commonly was *B. cinerea* isolated that the writers suspected it to be parasitic under the same conditions that several of the other fungi already discussed were found to be storage-rot producers.

Preliminary experiments were made first with raw sweet-potato blocks. It is not believed that the results from the use of raw sweet-potato blocks give a final proof of the parasitism of any organism, but they do give some indications of what may be expected when the potato itself is inoculated and exposed to similar temperatures. The raw blocks were cut and dropped into test tubes containing sweet-potato decoction, made according to the method described on page 339. These blocks were then inoculated with pure cultures of *Botrytis cinerea* and the tubes divided into five lots. One lot was placed in each of chambers 1 (1.12° C.), 2 (4.6° C.), 3 (6.15° C.), and 5 (9.4° C.) of the Altmann thermostat. This experiment was conducted with *B. cinerea* from two distinct sources. One strain (No. 3900) was isolated from sweet potatoes exposed to a temperature of 5° C. for several weeks and the other (No. 3940) from cabbage held in cold storage (0°–1° C.) for several weeks. In 16 days after inoculation all the plugs were rotted in all the chambers by both strains. The fungus fruited in the tubes. Controls held for the same experiment remained sound to the close of the experiment.

The above results were verified by an experiment in which potatoes were used, nine of which after inoculation (strain 3940) by the "well" method were put in each of compartments 1 (2.4° C.), 2 (3.56° C.), 4 (7.5° C.), 6 (13.9° C.), 9 (20.9° C.), and laboratory room. The results showed that this organism would decay sweet potatoes over a considerably wider range of temperatures than was the case with some of the other organisms which are virulent only at low temperatures. In fact, the rot did not progress as rapidly in the low temperatures as in temperatures a little higher. In other words, it required 30 days to completely decay the potatoes in chambers 1 and 2, while in chambers 4 and 6 the potatoes were entirely rotted in 20 days. In chamber 9 only two were completely rotted, and the remainder partially so in 23 days. In the laboratory room one potato was completely rotted and six partially so in 23 days;

the other remained sound. From this experiment it seems that medium temperatures are more favorable to *Botrytis cinerea* than the higher or lower ones.

Botrytis cinerea produces a grayish, soft, somewhat watery rot (Pl. 27, D). The tissue of the sweet potato pulls out in strings when broken apart. It has a somewhat starchy odor.

EPICOCCUM SP.

Although this fungus can not be regarded as of much economic importance, it was so often isolated from rotted sweet potatoes held at low temperatures that it can not be passed over without mention. It grows rather slowly and is probably able to cause decay only at such temperatures at which the competition of other fungi is reduced.

From a lot of sweet potatoes which had been thoroughly washed and then stored at 0°, 5°, and 10° C. *Epicoccum* sp. was about the only fungus isolated. Later a series of experiments were conducted in which sound potatoes were inoculated with pure cultures by the "well" method and exposed to the temperatures of chambers 3 (7.19° C.), 6 (14.4° C.), 9 (20.9° C.), and 11 (26° C.) of the Altmann thermostat. All the potatoes in chamber 3 were slightly to completely decayed in three weeks. In the other compartments they remained sound.

Epicoccum sp. produces a slow, firm rot. The tissue is rendered at first slightly yellowish followed by a reddish brown color (Pl. 27, E).

GIBBERELLA SAUBINETII

From the number of species of Fusarium that can be isolated from decayed sweet potatoes only a few have been shown to cause storage rots. Because of the almost omnipresence of some of the species of Fusarium an immense amount of work was done with these fungi. Strange to say, those species most frequently isolated are those which the writers were unable to prove parasitic or rot-producing organisms. On the other hand, those species shown to be capable of causing decay are, comparatively speaking, seldom found under natural conditions.

EXPERIMENT I.—Experimental work with this organism as a storage-rot fungus was started in 1914, when 75 potatoes after a thorough washing were divided into two lots, one of which was disinfected in formalin (1:200) for 30 minutes. Both lots were inoculated with *Gibberella saubinetii* (Mont.) Sacc. from pure culture by inserting spores and hyphæ into wounds. The treated lots only were wrapped in oiled paper. The two lots were then placed in storage, the temperature of which was maintained at about 2.0° C. They were left in storage for four months, when they were removed and isolations made from each potato. *G. saubinetii* was recovered in pure culture from only 20 per cent of the untreated and from 34 per cent of the treated lot. From the other

potatoes a miscellaneous lot of fungi was isolated, *Mucor racemosus* and *Mucor* sp. predominating. The sweet potatoes were a long time in storage at such a temperature, and, if not supported by other experimental data, these results would have little value. Even then they would hardly deserve to be included did it not form a link in the chain of experiments conducted with this organism and several species of Fusarium. All the controls in the above experiment were rotted, but none with *G. saubinetii.*

EXPERIMENT II.—On October 29, 1914, 40 sweet potatoes, after a thorough washing, were divided into two equal lots. One lot was disinfected for 30 minutes in a 1 to 200 solution of formaldehyde (40 per cent). The two lots were inoculated by inserting spores and hyphæ into a wound. The potatoes were then immediately immersed in a suspension of the spores in sterile water, after which they were wrapped in moistened filtered paper and then in oiled paper and placed in cloth bags. The two lots were stored near together in a sweet-potato storage house at Arlington Experimental Farm, Virginia. These potatoes were removed from storage March 11, 1915, and cultures made from those that were partially or completely decayed. Sixty-seven per cent of the treated lot remained sound; the others were from one-third to two-thirds decayed. *Gibberella saubinetii* was isolated in pure culture from 85 per cent of those that were decayed. Fifty per cent of the untreated lot (10 potatoes) remained sound and *G. saubinetii* was isolated from 90 per cent of those that were decayed. Thirty-five per cent of the controls (20 sweet potatoes) rotted slightly, but *G. saubinetii* was not isolated from any of these.

EXPERIMENT III.—A third lot (21 sweet potatoes), disinfected as above and wrapped but not immersed in a spore suspension, was stored in an Irish potato storage house. The temperature here was naturally lower than that of a sweet potato storage house, probably about 2.0° to 4.5° C. All these potatoes were more or less decayed and *Gibberella saubinetii* was recovered in pure culture from 67 per cent. The controls were also decayed, but *G. saubinetii* was not isolated from any.

EXPERIMENT IV.—In this experiment the sweet potatoes (40) were first placed in cold storage for one week and then inoculated by inserting spores and hyphæ into a wound, after which they were returned to cold storage. Before inoculation one lot was disinfected as above in formalin and wrapped. The potatoes were all decayed when removed from storage. *Gibberella saubinetii* was recovered from 90 per cent of the treated and 40 per cent of the untreated.

Gibberella saubinetii renders the sweet potatoes at first spongy in texture and brown in color. At a later stage, as moisture escapes, the tissue becomes firmer and finally hard and mummified. The brown color is later replaced by a pinkish-brown tint.

FUSARIUM CULMORUM

The experiments with *Fusarium culmorum* Wollenw. as well as *F. acuminatum*, to be discussed later, were for the most part carried out like those of *Gibberella saubinetii*.

EXPERIMENT I.—Eighty potatoes were thoroughly washed and divided into two lots. One lot was disinfected in formalin (1:200) for 30 minutes. After inoculation by inserting spores and hyphæ the treated lot was wrapped in oiled paper and stored at 2.0° C. When they were removed from storage, cultures were made from each potato. *F. culmorum* was isolated in pure culture from 95 per cent of the treated and 25 per cent of the untreated lot. The other potatoes were decayed, but a miscellaneous lot of fungi, of which *Mucor racemosus* and *Mucor* sp. predominated, was isolated. *F. culmorum* was not isolated from any of the controls. A more detailed discussion of the method may be found under experiment I of *Gibberella saubinetii*.

EXPERIMENT II.—The method of manipulation in this experiment is identical with that of Experiment II of *Gibberella saubinetii*. Two lots of potatoes (20 in each lot) were removed from the sweet-potato storage house on March 11 and cultures made from each decayed or partially decayed potato. Sixty per cent of the treated lot (formalin, 1:200, 30 minutes) were one-half to two-thirds decayed, and 80 per cent of the untreated were partially to completely so, and *F. culmorum* was isolated from all in pure culture. The other inoculated potatoes and the controls remained sound.

EXPERIMENT III.—For method see Experiment III of *Gibberella saubinetii*. This experiment differs from Experiment III above in that there were two lots, only one of which was treated. All these potatoes were decayed when removed from storage, and *F. culmorum* was isolated from 100 per cent of the treated potatoes and 95 per cent of those not treated. While most of the controls were decayed, *F. culmorum* was not isolated from any.

EXPERIMENT IV.—For method see Experiment IV of *Gibberella saubinetii*. These sweet potatoes were kept in storage from September 9 to January 18, when they were removed and cultures made from those partially or completely decayed. Ninety-five per cent of the treated and 80 per cent of the untreated potatoes were rotted with *F. culmorum* and the causal organism recovered in pure culture from each.

EXPERIMENT V.—Chambers 4 (10.6° C.), 5 (13.5° C.), 7 (16.9° C.), 9 (20.5° C.), 10 (21.2° C.) of the Altmann thermostat and laboratory room (23° C.) were used for this experiment. The potatoes were thoroughly washed, then disinfected in mercuric chlorid (1: 1,000) for 10 minutes. They were inoculated on November 6 by inserting spores and hyphæ into a wound at the end, after which they were wrapped in moistened filter paper and then in oiled paper. On January 3 they were removed from

the different chambers and cultures made. All the potatoes in chamber 4 (10.6° C.) were decayed, and *F. culmorum* was recovered in pure culture. Only 10 per cent were decayed in chambers 5 (13.5° C.) and 7 (16.9° C.), and *F. culmorum* was isolated from all. The potatoes in the other chambers and all the controls remained sound.

F. culmorum produces a distinctively characteristic rot and when once seen would never be confused with any of the other rots except that caused by *F. acuminatum*, from which it does not differ macroscopically. The organism decays the sweet potato slowly, requiring three to six weeks to complete its entire destruction. The tissue is rendered spongy but not watery. In the early stages the tissue of the host is a faint reddish brown, which turns later to a carmine-red or maroon. As the potato dries out, some of the deep color is lost, and in the mummified stage it becomes a beautiful pink.

FUSARIUM ACUMINATUM

EXPERIMENT I.—The method is identical with that used in Experiment I of *F. culmorum*. When these sweet potatoes were removed from storage, all of the treated potatoes and 75 per cent of those not treated were decayed, and *F. acuminatum* Ell. and Ev. emend. Wollenw. was recovered from each in pure culture. The only other fungi isolated were *Mucor racemosus* and *Mucor* sp.

EXPERIMENT II.—The method is the same as in Experiment II of *Gibberella saubinetii*. When these sweet potatoes were removed from storage, only a small percentage had decayed. *F. acuminatum* was recovered from 5 per cent of the treated lot and 17 per cent of those not treated. *F. oxysporum* was recovered from a few other potatoes.

EXPERIMENT III.—The same method was used as that in Experiment III of *Gibberella saubinetii*. When these sweet potatoes were removed from storage, *F. acuminatum* was isolated from 75 per cent of the treated and 90 per cent of the untreated. The only other fungi isolated were *Mucor racemosus* and a species of Penicillium.

EXPERIMENT IV.—The method was the same as that used in Experiment IV of *Gibberella saubinetii*. All the sweet potatoes were decayed, and *F. acuminatum* was isolated in pure culture from 95 per cent of both the treated and untreated. Controls were held for all these experiments and while, as might be expected, some of them were decayed, for which several fungi were responsible, *F. acuminatum* was not isolated from any.

TRICHODERMA KONINGI

We owe our first knowledge of a storage-rot of sweet potatoes caused by *Trichoderma koningi* Oud. to Cook and Taubenhaus (*8*), who found it associated with ringrot and softrot. It probably falls into the class with several other organisms of minor importance which can cause decay

of the roots when removed from the competition of other fungi. The writers have seldom isolated the fungus, and in no case has it ever been obtained from wounds or decayed tissue where it seemed the primary cause of the rot. Cook and Taubenhaus were able to infect sweet potatoes artificially by inoculating them with pure cultures of *T. koningi*. A rot differing somewhat from that caused by *T. koningi* in character was produced by artificial inoculation with *T. lignorum* (Lode.) Harz. The latter fungus, however, was not isolated from sweet potatoes, but was obtained by the investigators mentioned above from Dr. Thaxter. Both of these species of Trichoderma are widely distributed fungi, and *T. koningi* is especially common in the soil. Therefore it is not surprising to find it associated with other fungi in the rotted tissue of sweet potatoes.

The symptoms caused by *T. koningi* are described by Taubenhaus and Manns (*37, p. 24*) as follows:

> In the earliest stages the spots are circular and of a light brown color, with a tendency to wrinkle. The flesh is hard and watersoaked, brown in color, with a black zone in the region between the healthy and diseased tissue. The spot enlarges in all directions and eventually destroys the entire root.

DISCUSSION OF RESULTS

In the foregoing pages 17 organisms have been found to bear some relation to storage-rots of sweet potatoes. Naturally, some of these are of minor importance, but perhaps largely because the conditions suitable to their needs are not usually maintained in the storage house. In fact, there is not a single organism here discussed which did not exhibit a preference for certain evironmental conditions. Such fungi as *Rhizopus nigricans* and *Sphaeronema fimbriatum* find conditions suitable for them to rot sweet potatoes at the usual storage temperature (50° F.) or at higher temperatures. On the other hand, such organisms as *Mucor racemosus*, *Botrytis cinerea*, and others find such tenmperatures unsuited to their needs and bring about destruction of the host only at temperatures considerably lower than that usually recommended for the storage of the crop.

Humidity was shown to play an important part. *Rhizopus nigricans* requires a relatively high humidity until it has once started, after which it will complete the destruction of the potato in an atmosphere almost entirely free of moisture. *Sphaeronema fimbriatum* also grows better in the presence of abundant moisture. *Diplodia tubericola*, on the other hand, will grow in a relatively dry atmosphere from the outset. *R. nigricans* completes the destruction of the potato in a few days, while *D. tubericola*, *Diaporthe batatatis*, and others are slow growers, requiring from three to eight weeks.

Although the writers have discussed at some length organisms which thrive best at temperatures below that recommended for the storage of the crop, sight must not be lost of the fact that the recommendations for

storage are not universally followed and not necessarily trusted by everyone. We have occasionally found sweet potatoes stored with Irish potatoes and in cellars with cabbage, turnips, and other root crops for which a low temperature was required. In the South most of the sweet potatoes are stored in earth banks, and often when these are opened a considerable percentage of all of the crop is found decayed. The potatoes about the edges and on top in some of these banks are frozen, indicating that probably the entire lot had been exposed to a low temperature perhaps for a considerable length of time. Furthermore, sweet potatoes are taken from storage during the winter and shipped distances requiring from 3 to 12 days or more for them to reach the market. Since most of these shipments are to the northern and eastern markets, they may be, and frequently are, subjected to a low, if not freezing, temperature. After reaching the terminals they may be subjected to lower temperatures for a considerably longer period by the usual methods of handling. An examination of such material showed that they often arrive in bad condition. Many of the potatoes are rotted or partially so and unsalable. A study of such material, as well as material taken from cellars and Irish potato storage houses, shows that they are not decayed by *Rhizopus nigricans* and some of the other well-known storage-rot fungi, but by some of the forms discussed above, requiring low temperatures, as, for example, *Mucor racemosus* or *Botrytis cinerea*. So, bearing in mind the fact that sweet potatoes, even in storage, during transportation, and at the terminals may be subjected to the temperatures suited to some of these forms, it will be readily understood that the loss caused by these organisms may be considerable. Moisture is, of course, essential for those forms which are so much in evidence at low temperatures. In the banks there is little, if any, provision made for the escape of moisture. In cellars and in storage houses designed primarily for other purposes it is inadequately provided for. Sweet potatoes are generally shipped in barrels or bushel baskets and the moisture may accumulate in car-load shipments, especially if the temperature is low. The optimum comditions for some of these fungi are, therefore, unavoidably provided.

The facts presented as to the specific requirements of various fungi in general are not new. Link (*25*) has shown that while both *Fusarium oxysporum* and *F. trichothecioides* Wollenw. can produce tuber-rots and wilt of the Irish potato, the optimum temperature of *F. oxysporum* is higher than that of *F. trichothecioides*. Similar data has been published by Brooks and Cooley (*4*), who found from a study of the temperature relations of the apple storage-rots that the optimum temperature requirements of the various fungi differed greatly. *Sphaeropsis malorum* produced no evident rot at 15°, nor did the species of Penicillium and Neofabraea at 10° at the end of a week, while *Sclerotinia cinerea* produced a measureable rot at 5° in one week and at 0° in two weeks. The optimum temperature of *Neofabraea malicorticis* was found to be 20°, *F. radicicola* 30°, all the

other fungi 25°. The range of optimum temperature requirements for apple-rot fungi is even greater than for the storage-rot fungi of the sweet potato. That cool weather is required for infection of the Irish potato by the lateblight fungus, *Phytophthora infestans*, is well known. On the other hand, Gilman (*11*) concludes that relative high temperatures are required for infection of cabbage by *F. conglutinans* and Tisdale (*38*) arrives at similar conclusions for the infection of flax by *F. lini*. Other references might be made to show that the various fungi have different temperature requirements. On this point no generalizations can be made.

OTHER FUNGI ISOLATED AND STUDIED

A considerable number of other fungi, mostly species of Fusarium has been isolated and studied—namely, *F. batatatis* Wollenw., *F. hyperoxysporum* Wollenw., *F. radicicola* Wollenw., *F. caudatum* Wollenw., *F. solani* (Mart.) Sacc., *F. incarnatum* (Rob.) Sacc., *F. orthoceras* Appel and Wollenw., *F. orthoceras* var. *triseptatum* Wollenw., *F. oxysporum* Schlect., *Nectria ipomoeae* Hals., and an undetermined species of Mucor. These fungi have all been subjected to the same tests of parasitism as those classed as storage-rot organisms.

Preliminary experiments were conducted in which sweet potatoes were inoculated at the end or in some wound and then confined in a moist chamber, both wrapped and not wrapped in moist filter paper and then in oiled paper. They were inoculated by wounding and then dipped in spore suspensions and confined in moist chambers but without result. Chilling the potatoes by exposing them to cold-storage temperatures (2.0° C.) for a week and then inoculating in the usual way did not yield results. Soaking in water before inoculation was without effect. A large number of sweet potatoes have been inoculated, after which they were divided up into several lots and exposed to the temperatures of the different chambers of the Altmann thermostat. In no case were the results consistent enough to warrant the conclusion that any of these organisms would cause decay at the temperatures used.

Fusarium batatatis and *F. hyperoxysporum* are the two species well known as the cause of stemrot (*23*) of sweet potatoes. These two organisms frequently invade the fibrovascular bundles of the roots, often extending entirely through the potato. To know whether these organisms caused storage-rots is of considerable importance in view of their prevalence and destructiveness to certain varieties in some parts of the country. Roots naturally infected have been gathered and stored in bushel baskets in the storage houses with the other potatoes, and in every case they kept just as well as healthy potatoes. Naturally infected roots, after a thorough washing, have been wrapped in wet filter paper and then in oiled paper and subjected to the temperatures of the different chambers of the Altmann thermostat. Not in a single chamber did any of the potatoes rot. The writers conclude from these results that these

two organisms do not cause storage-rots. However, another organism, *F. oxysporum*, which might easily be mistaken for *F. batatatis* but hardly for *F. hyperoxysporum*, is one of the most common inhabitants of decayed sweet potatoes. In most storage houses it is common to find potatoes decayed at the end for a distance of ½ to 1½ inches, the tissue being brown and firm and emitting a pleasant aromatic odor (Pl. 27, F). From such decayed ends and from certain surface lesions and wounds *F. oxysporum* is the most common species of Fusarium isolated with any degree of regularity. Occasionally other fungi may be isolated, as, for example, *Plenodomus destruens*, *Nectria ipomoeae*, or *F. orthoceras*. Although it is the belief of the writers that *F. oxysporum* does cause an endrot of stored sweet potatoes we have consistently failed to obtain proof of it by any of the methods employed.

Nectria ipomoeae Hals. is another fungus frequently found on decayed sweet potatoes. It was first thought by Halsted to be the cause of the stemrot of sweet potato and eggplant, but was later shown (*22*) to be only a saprophyte. Since it was frequently isolated from rotted sweet potatoes, *N. ipomoeae* was suspected of causing a storage-rot, but like many of the other organisms studied, it consistently failed to give positive results by any of the methods tried.

Fusarium caudatum is not a common inhabitant of decayed sweet potatoes. It was originally isolated from a number of sweet potatoes sent from Clemson, S. C., by Prof. H. W. Barre. Prof. Barre said that several hundred bushels had been thrown out of a storage house similarly decayed. The decayed potatoes were brown in color, firm in texture, and had a very pleasing aromatic odor. We were unable by any of the methods tried to prove this organism the cause of a storage rot. None of the other forms with the exception of *F. radicicola* are very common. This species of Fusarium was frequently isolated from decayed potatoes and from the wounds and lesions of field material, especially from the South. However, in our experiments it has given no evidence of being a storage-rot producer. It is a common soil saprophyte and probably a secondary invader.

At different times other fungi have been isolated with which no experiments have been conducted. They were so rarely met with that inoculation experiments did not seem justified. Among others the following may be mentioned: *Zygorhynchus* sp., *Penicillium* sp., *Melanospora* sp., *Trichosporium* sp., *Ceratostoma* sp., *Sporotrichium* sp., *Pestalozzia* sp., *Aspergillus niger* Von Tiegh, *Sclerotium rolfsii* Sacc., *F. vasinfectum* Atk., *Cephalothecium* sp., *Neocosmospora vasinfectum* Atk,., *Verticillium cinnabarinus*, *Acromoniella* sp., *Macrosporium* sp., *Actinomyces* sp., and others.

SUMMARY

(1) Storage-rots of sweet potatoes are estimated to cause a loss of many million dollars annually.

(2) Seventeen different fungi were found responsible for the decay of sweet potatoes in storage.

(3) A few of these fungi—viz, *Rhizopus nigricans*, *Sphaeronema fimbriatum*, *Diplodia tubericola*, *Diaporthe batatatis*, *Plenodomus destruens*, *Sclerotium bataticola*, and *Monilochaetes infuscans* are responsible for the most of the loss.

(4) The following fungi cause losses in storage under favorable conditions and are designated as the minor rot-producing fungi: *Mucor racemosus*, *Alternaria* sp., *Penicillium* sp., *Botrytis cinerea*, *Epicoccum* sp., *Gibberella saubinetii*, *Fusarium culmorum*, *F. acuminatum*, and *Trichoderma koningi*.

(5) Most of these fungi are weak wound parasites capable of causing decay of sweet potatoes only under particularly favorable conditions. *Rhizopus nigricans*, which probably causes more loss than any other organism, would not consistently infect sweet potatoes without first germinating the spores. When the germinated spores and decoction in which they were suspended were poured into a "well" in the potato, infection would usually follow.

(6) After infection had once started, *Rhizopus nigricans* would complete the destruction of a potato in an atmosphere almost entirely lacking moisture.

(7) There was great variation in the time required for the different fungi to completely decay a potato. For *Rhizopus nigricans* from three to five days were required. *Diplodia tubericola*, *Diaporthe batatatis*, and others required three to eight weeks under similar conditions.

(8) Most of the fungi required a considerable amount of moisture. In fact, wrapping in moistened filter paper was often necessary after inoculation by the "well" method. *Diplodia tubericola*, on the other hand, grows as well or better in a humidity of about that of the laboratory room.

(9) The optimum temperature of the different organisms varied considerably. The optimum for *Rhizopus nigricans* was comparatively high, but it would decay the potatoes over a considerable range of temperatures. On the other hand, *Mucor racemosus*, *Fusarium culmorum*, *F. acuminatum*, and others had a relatively low optimum.

(10) Some of these storage-rot fungi are also the cause of field diseases of sweet potatoes. Such are *Sphaeronema fimbriatum*, *Plenodomus destruens*, and *Monilochaetes infuscans*.

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PLATE 21

A.—Sweet-potato softrot, caused by *Rhizopus nigricans*. Only three to four days are required to rot completely the potato. If the atmosphere is sufficiently moist and the epidermis is broken, the sporangia develop superficially in great abundance.

B.—Sweet-potato ringrot, caused by *Rhizopus nigricans*. Ringrot differs from softrot only in that the decay extends around the potato at one or more places between the two ends. The causal organism may or may not advance toward the two ends, finally completing the destruction of the potato.

A
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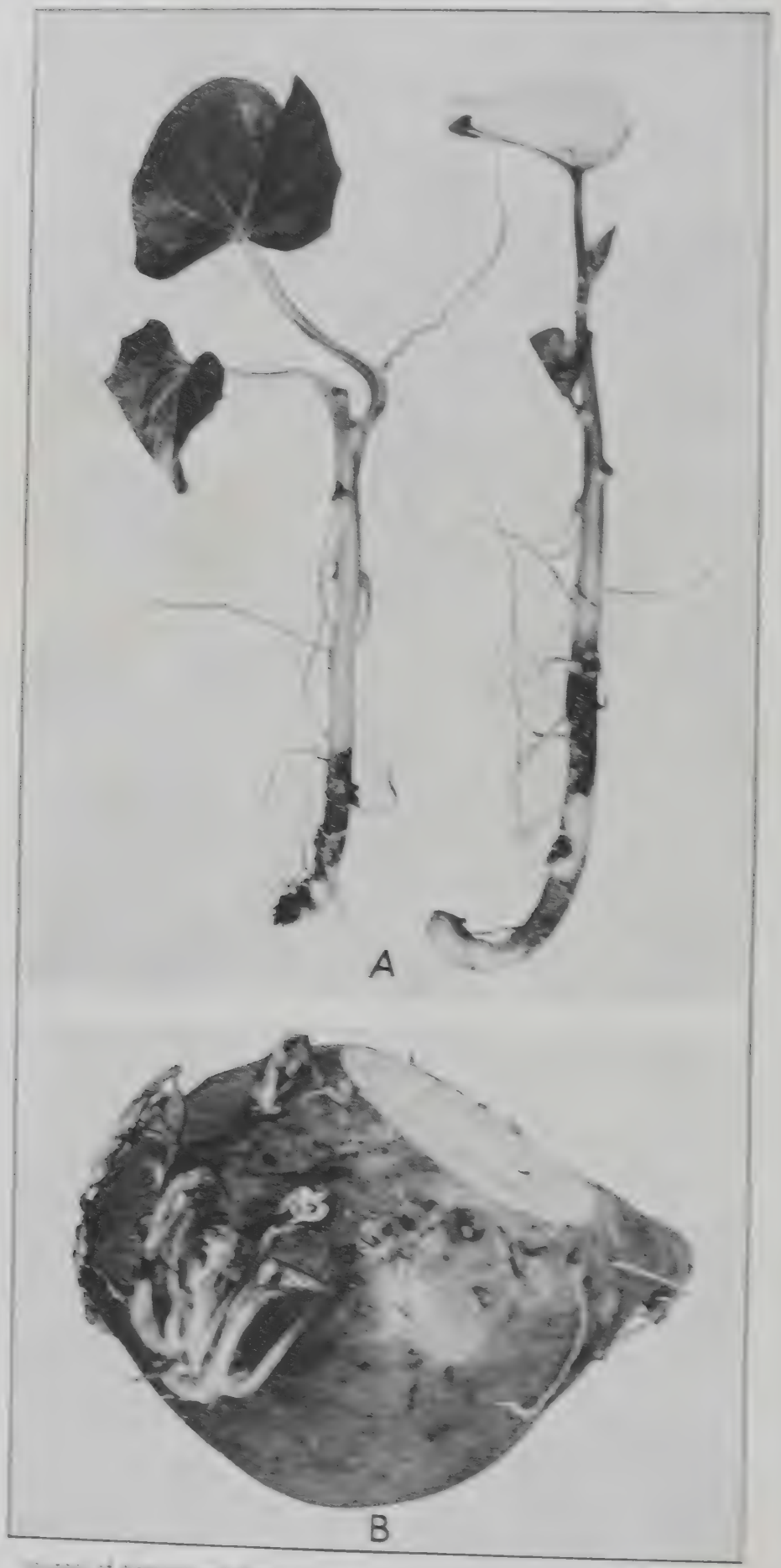
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PLATE 22

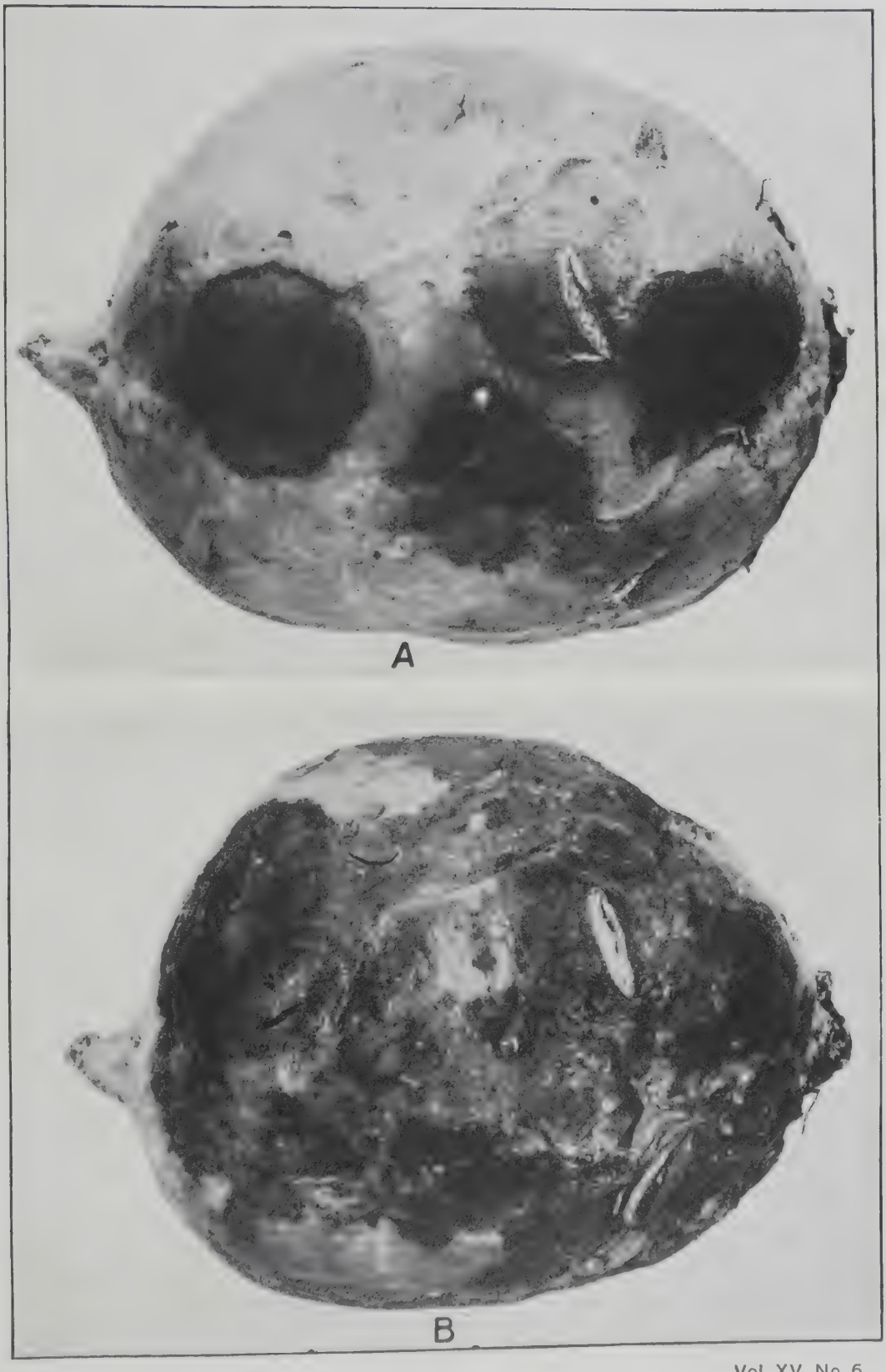
A.—Flowers of young potato plant attacked by *Phytomyza ferruginea*. Note the black specks on the underground part of the stem. The larval burrow gave rise to the symptoms of blackening pictured in detail. The use of infected soil in the kennel will cause similar infection.

B.—Flowers of a healthy young potato plant. Note the young sprouts not yet damaged, the ground having remained free from the fungus.

PLATE 23

A.—A sweet potato with four blackrot spots caused by *Sphaeronema fimbriatum* taken from a storage house in November.

B.—The same sweet potato shown in A after being kept in an ice box for two months. The temperature of the ice box was about the same as that recommended for the storage of sweet potatoes. Note that the spots have enlarged and united so as to involve nearly the entire potato.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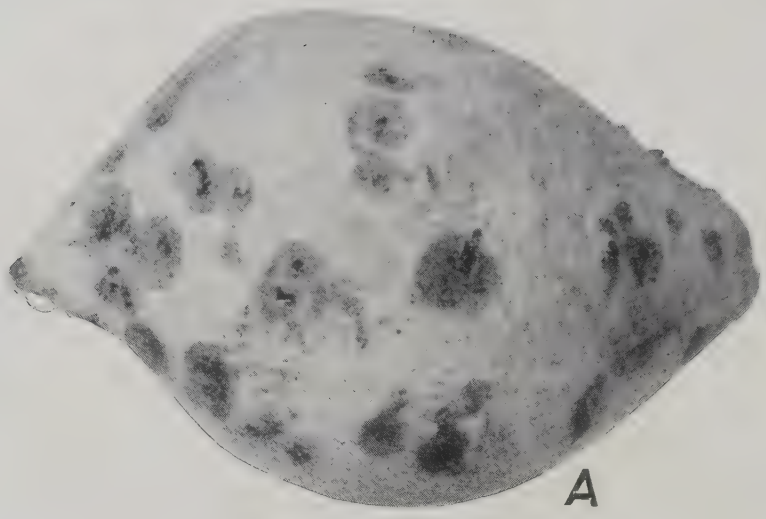

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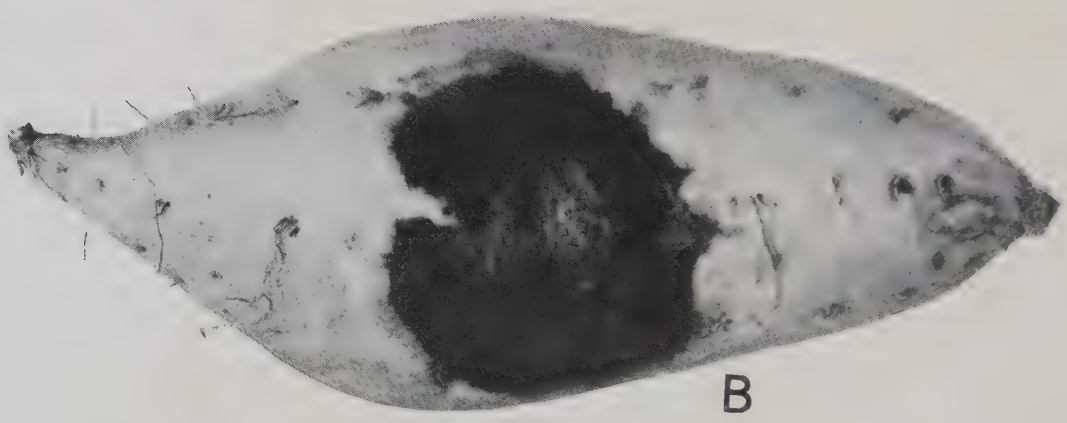

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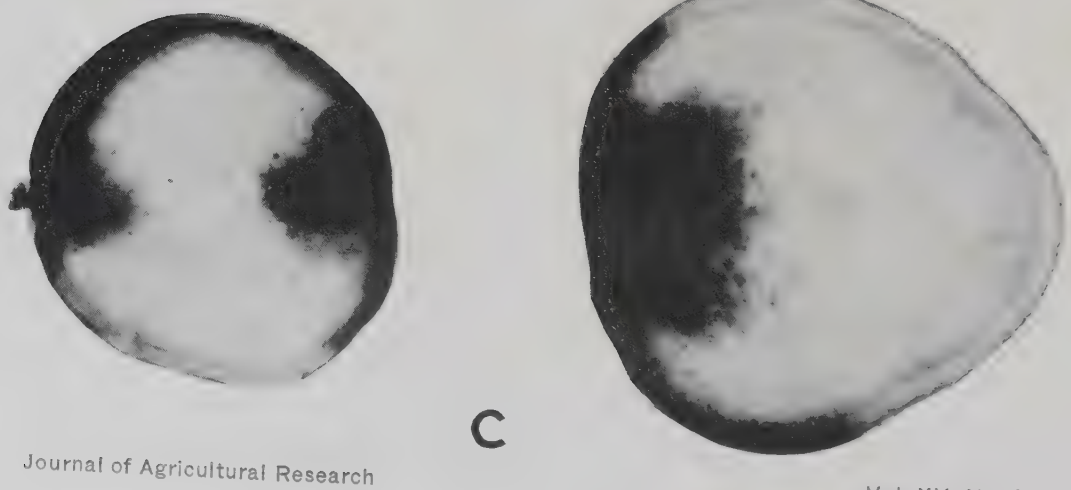

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PLATE 24

A.—An originally healthy sweet potato sprayed with the spores of *Sphaeronema fimbriatum* and confined in a moist chamber. The infections take place mostly through small rootlets or through wounds.

B.—A typical blackrot spot on a sweet potato as usually found at digging time or in storage.

C.—Cross sections of blackrot sweet potatoes, showing the depth to which the fungus will sometimes penetrate. Pure cultures of *Sphaeronema fimbriatum* were obtained from the deepest portion of the rot of these two sections.

PLATE 25

A.—A sweet potato decayed by the Java blackrot fungus *Diplodia tubericola*. Numerous pycnidia may be seen on the surface.

B.—A sweet potato decayed by the dryrot fungus *Diaporthe batatas*. Note the dry, shrunken appearance of the potato.

A
B

A
B
C

PLATE 26

A.—A section through a sweet potato partially decayed by the foxrot fungus, *Plenodomus destruens*.

B.—Sweet-potato scurf, caused by *Monilochaetes infuscans*. The number of infections is shown by the black spots on the surface.

C.—A sweet potato entirely covered with scurf. In infections as bad as this the potato shrinks and finally dries up, becoming eventually worthless.

PLATE 27

A.—A cross section of a sweet potato decayed by *Mucor racemosus* at a temperature of 5° C. The mottled appearance is characteristic of rot caused by this fungus at low temperatures.

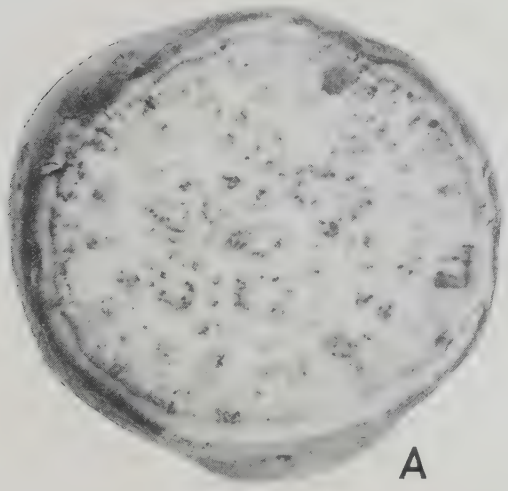
B.—A longitudinal section of a sweet potato decayed by *Alternaria* sp. The tissue becomes a very dark brown to nearly black.

C.—A portion of a sweet potato probably decayed by *Penicillium* sp. Note the numerous cushions of the fungus on the surface.

D.—A cross section of a sweet potato showing the characteristic appearance of the rot caused by *Botrytis cinerea*.

E.—A cross section of a sweet potato almost completely decayed by *Epicoccum* sp. The netted string-like appearance, also the yellowish color produced at some stages in the progress of the rot, is characteristic of the decay caused by this fungus.

F—A longitudinal section of a partially decayed sweet potato. This so-called end-rot is quite common in storage. *Fusarium oxysporum* is generally isolated from such decayed tissue. Although this fungus is believed to be the cause of endrot, inoculation experiments have never given consistent results.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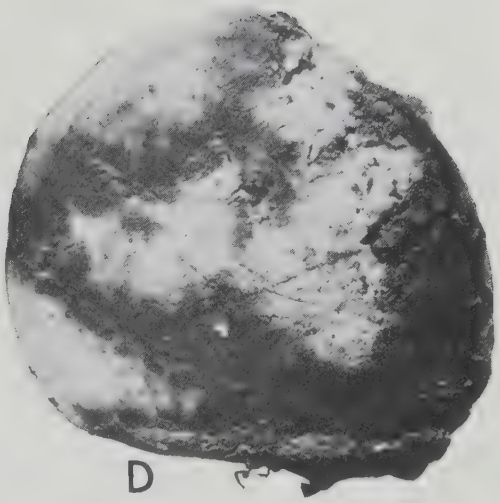
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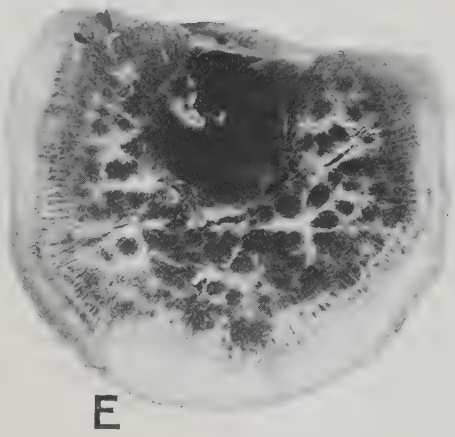
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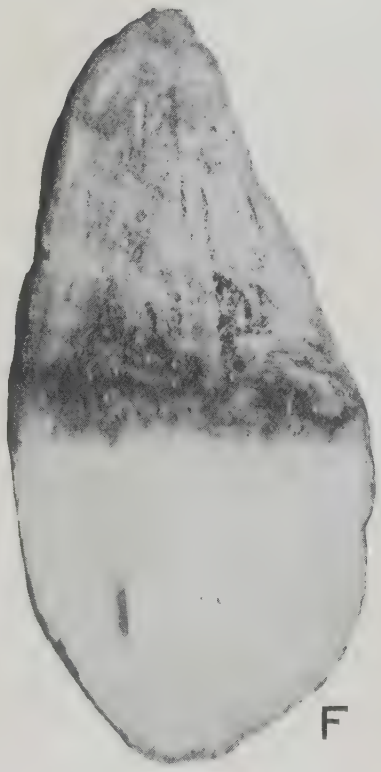
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JOURNAL OF AGRICULTURAL RESEARCH

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PHYSIOLOGICAL STUDIES OF NORMAL AND BLIGHTED SPINACH[1]

By RODNEY H. TRUE, OTIS F. BLACK, JAMES W. KELLY, H. H. BUNZELL, LON A. HAWKINS, SAMUEL L. JODIDI, and EDWARD H. KELLOGG, *Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTORY STATEMENT

For some years the growing of spinach (*Spinacia oleracea*) in the trucking sections of Norfolk, Virginia, has been seriously affected by a disease known as "spinach-blight," which is marked by a dwarfing of the affected plants, with a change of color from dark to yellowish green, and the development of a sweet taste and bitterness when the leaf is folded, similar to that seen in a mature tobacco leaf.

This disease has been shown by McClintock and Smith (*29*)[2] to belong to the "mosaic" group. It is therefore a "virus disease" readily communicable from blighted to healthy plants by contact, by injection of plant extracts, and especially by aphids. These insects are responsible for the rapid spread of the disease in the field.

Earlier work by Harter (*16*) and others on the malnutrition of truck crops has led to the belief that this spinach trouble was attributable to the lack of lime and humus, with excessive acidity of the soil, and the work reported in these papers was begun in the hope of throwing light on the abnormal physiological reactions observed. In carrying out these plans, laboratory investigations were made of the ash, carbohydrate, and oxidase contents of both normal and blighted plants, as well as a more fundamental study of the nitrogen metabolism.

Since the nutrition of the plants is closely connected with the condition of the soil in which they grow, and since it has been suggested that the occurrence of the disease might in some degree be influenced by soil conditions (*16*, *17*), it was deemed necessary to take these possible factors into account. The results of an examination of field conditions by Dr.

[1] The investigations here presented bear on different phases of the same problem, although carried out by different workers. Since different men are responsible for the results presented, requiring as they do different types of technic and different lines of special interest, the results are presented in separate chapters in which both the responsibility and the credit of authorship are separately indicated.

[2] Reference is made by number (italic) to "Literature cited," pp. 405–408.

Jay A. Bonsteel, Soil Specialist of the Bureau of Soils, seemed to exclude faulty drainage from the list of possible causes.

The studies reported in this series of papers were carried out in cooperation with the Office of Cotton, Truck, and Forage-Crop Disease Investigations and with the Virginia Truck Experiment Station. From both organizations highly appreciated help was received. The writers are especially indebted to Mr. J. A. McClintock, then Pathologist at the Virginia Truck Experiment Station. He took notes on field conditions, selected and prepared authentic material for laboratory study, and aided in many other ways.

It is believed that this biochemical study of a "mosaic" disease will perhaps have fully as great an interest for plant pathologists as for physiologists.

RODNEY H. TRUE,
Physiologist in Charge of Plant Physiological and Fermentation Investigations.

ASH CONTENT IN NORMAL AND IN BLIGHTED SPINACH

By RODNEY H. TRUE, *Physiologist in Charge*, OTIS F. BLACK, *Chemical Biologist*, and JAMES W. KELLY, *Laboratory Technician, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry.*

In view of the fact that disease conditions not infrequently profoundly modify the demands of the organism for mineral constituents, a study of the ash was undertaken in the hope of getting some light on the nature of the abnormal conditions set up in the spinach by the disease-producing agent.

Typical normal and diseased material was selected by Mr. McClintock from the fields of truck growers living near the Truck Experiment Station. The plants were collected in February, 1915, the roots and stems being carefully dug out by means of a spade. The adhering soil was immediately washed off as well as could be done in the field, and the plants, well wrapped up, were taken to the laboratory, where they were weighed and spread out to dry.

After they had become thoroughly air-dry, the samples were ashed in an electric oven at a temperature of about 700° C. at a cherry-red color. After the quantity of total ash had been ascertained, its constituents were determined according to the methods of the Association of Official Agricultural Chemists.[1]

TABLE I.—*Ash content of healthy and diseased spinach plants*

[Calculated as percentage of total ash]

Constituent.	Jones farm.				Whitehurst farm.			
	Healthy.		Diseased.		Healthy.		Diseased.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.39	6.70	18.23	9.74	21.41	7.50	16.68	8.54
Silicon dioxid (SiO_2)	23.89	20.70	35.66	19.76	32.41	18.00	35.99	23.03
Red manganese oxid (Mn_3O_4)	.16	.31	.24	3.38 (?)	.10	.90	.27	1.22
Calcium oxid (CaO)	6.48	4.90	11.88	7.01	4.62	6.07	11.63	9.60
Magnesium oxid (MgO)	3.47	5.35	4.61	9.96	4.43	4.90	4.52	9.19
Potassium oxid (K_2O)	32.06	14.06	23.91	35.19	26.38	12.40	22.60	} 59.95
Sodium oxid (Na_2O)	10.03	24.64	11.32	5.35	13.70	24.47	10.99	
Sulphur trioxid (SO_3)	3.14	2.70	1.87	2.94	3.00	6.10	1.99	3.55
Phosphorus pentoxid (P_2O_5)	6.71	13.40	6.36	15.15	6.19	14.20	4.05	13.55
Aluminium oxid (Al_2O_3)	2.59	2.69	4.41	2.51	4.03	2.37	3.49	1.06
Ferric oxid (Fe_2O_3)	.51	.93	.56	.96	.67	.92	.50	1.78

The results are shown in Tables I and II. In Table I the total ash is given as percentage of the total weight, air-dry, and the constituents

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

are given in percentages of the total ash. In Table II the total ash, and likewise the constituents, are calculated in percentages of the dry weight of the plant material.

TABLE II.—*Ash constituents of healthy and diseased spinach plants*

[Calculated as percentages of dry weight]

Constituent.	Jones farm.				Whitehurst farm.			
	Healthy.		Diseased.		Healthy.		Diseased.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.39	6.70	18.23	9.74	21.41	7.50	16.68	8.54
Silicon dioxid (SiO_2)	4.63	1.39	6.50	1.92	6.94	1.35	6.00	1.96
Red manganese oxid (Mn_3O_4)	.031	.021	.043	.33	.021	.067	.045	.104
Calcium oxid (CaO)	1.26	.33	2.17	.68	.99	.46	1.94	.82
Magnesium oxid (MgO)	.67	.36	.84	.97	.95	.37	.75	.78
Potassium oxid (K_2O)	6.22	.94	4.36	3.43 (?)	5.65	.93	3.80	5.12
Sodium oxid (Na_2O)	2.40	1.65	2.06	.52	2.93	1.84	1.83	
Sulphur trioxid (SO_3)	.61	.18	.34	.29	.64	.46	.33	.30
Phosphorus pentoxid (P_2O_5)	1.30	.90	1.16	1.48	1.33	1.07	.68	1.16
Aluminium oxid (Al_2O_3)	.50	.18	.80	.24	.86	.18	.58	.09
Ferric oxid (Fe_2O_3)	.122	.062	.102	.093	.143	.069	.083	.152

While the quantity of total ash is not strikingly different in normal and in diseased material, the normal tops in all cases seem to be a little richer than the diseased tops, whereas the roots of the diseased plants have somewhat more ash than the normal roots. The great excess of ash in the leaves over the roots is in agreement with the general rule and is seen in both kinds of material.

It is interesting to note that spinach leaves have been found by others to contain an unusually large quantity of total ash, belonging in the same class as tobacco leaves (*Nicotiana tabacum*), hop leaves (*Humulus lupulus*), head lettuce (*Lactuca sativa*), forage-beet tops (*Beta vulgaris*) and *Elodea canadensis* in containing from 16.4 to 20 per cent of total ash. Wolff (*49, p. 141–150*) reports the average for spinach to be 16.48 per cent. The writers find the average of normal samples taken from two fields to be 20.4 per cent, the blighted 17.45 per cent. Thus, the tops of the normal plants are markedly richer in total ash than are those of the diseased plants. This relation is reversed in the roots, the blighted plants averaging 9.14 per cent, in comparison with 7 per cent in the normal material.

Concerning the individual constituents present, a number of points are worth noticing. The case of the silica content is one of especial interest. Although all usual precautions were taken to remove adhering soil, the silica present makes up one of the chief components of the ash, both in leaf and root. As may be seen in Table II, the tops contain several times

as much as the roots in all samples. The proportion of silica present seems to be little affected by health or disease either in tops or roots.

It is interesting to note that in the analyses reported by Wolff (*49, p. 141–150*), the average silica content of this plant, presumably of the tops, is but 4.52 per cent of the total ash against 28.15 per cent found here, and but 0.745 per cent of the total dry weight of the plant against 5.78 per cent found in the normal Norfolk tops. It would be interesting to know in how far the silica content of spinach varies with the locality in which it is produced. A high silica content seems in general to characterize the grasses and grains and not such succulent vegetables as spinach. In this material, however, we have a quantity present equaling that characteristic of the grains (*49*).

In this connection it is interesting to note Bertrand's (*5*) conclusion that the oxidase activity of plant tissues is related to the manganese content. Since the oxidase relations of normal and blighted spinach are dealt with in a separate paper (p. 377), it is sufficient to state here that in general the higher manganese ash content seems to accompany the stronger oxidase reaction in agreement with Bertrand's observations.

The tendency of calcium to become localized in the leaves in greater quantity than in the roots is seen in both normal and blighted samples. While in the normal material the average proportion of the total ash made up by calcium is about the same in tops and roots, in the diseased plants the greater proportion is clearly found in the tops. When the actual quantity of calcium present in a unit of dry weight is considered, the tops are seen to carry more than three times as much as the roots in both normal and blighted material and the diseased plants contain nearly twice as much calcium as the corresponding structures of the normal plants. There is here then a tendency of the diseased plants toward increased accumulation of calcium in both tops and roots. It may be noted in passing that according to the analyses given by Wolff (*49, p. 141–150*) spinach belongs among those plants which absorb calcium in rather limited quantities, like many of the common legumes. This conclusion is confirmed by the results here given.

Magnesium is present in both roots and tops of the blighted plants in nearly double the proportion of the total seen in the corresponding parts of normal plants and in both types of material forms a larger proportion of the ash of the roots than of the tops.

When considered with reference to the actual quantity of magnesium present in a given weight of dry material, that seen in the tops is about the same whether diseased or normal, the quantity seen in normal roots being less than that seen in roots of blighted plants.

The magnesium content found here is roughly about one-half that reported by Wolff (*49*), who lists spinach among the plants which absorb relatively large quantities of this substance.

One of the most striking features of this material, normal as well as blighted, is the high potassium content. While Wolff reports spinach as containing 2.729 per cent of potash, calculated on the dry weight of the material, the average percentage found in the normal tops here is 5.93 per cent and 4.08 per cent in the blighted tops. The roots seem to contain less than the tops both in health and in disease, but there seems to be more in the diseased than in the healthy roots.

Sodium, which is present in less quantity than potassium, seems to be more uniformly distributed throughout the plants. Like potassium, it is usually more abundant in the tops than in the roots in both healthy and diseased samples. Sodium is present in less quantity than potassium in the tops, but in some cases this relation is reversed in the roots. Wolff (*49*) reports 5.816 per cent of sodium, a quantity, presumably in the tops, about double that seen here in the normal tops. In any case, spinach seems to absorb an unusual quantity of sodium, betraying clearly its halophytic tendencies.

The sulphate radical here, as in most plants, is rarely absorbed in large quantity. According to Wolff (*49*), it reaches 1.113 per cent of the dry weight of the plant material. Here the quantity found in the normal tops is less than half of that amount, which in turn is about double that found in the blighted tops. The roots are poorer than the corresponding tops in each individual case.

Phosphate absorption is not heavy in spinach at the stage in which the disease appears, but seems to be influenced by the blight. In the tops the average normal phosphate content is 1.31 per cent of the dry weight of the plant, against 0.92 per cent in the blighted tops. This relation is completely reversed in the roots, the normal samples containing 0.98 per cent, against 1.32 per cent in the blighted roots.

These results, which agree fairly well with Wolff's data, place spinach with head lettuce and cauliflower hearts (*Brassica oleracea botrytis*) near the top of the list of leafy vegetables in the quantity of phosphates absorbed.

Aluminium, rarely absorbed in great quantity, is present in spinach in small amounts. The normal and blighted tops contain alike nearly 0.7 per cent, calculated on dry weight, while the roots in samples of both kinds agree in containing about 0.18 per cent each. According to Berthelot and Andre (*4*), the roots of plants usually contain more aluminium than the leaves.

It is probable that the material here studied is unusually high in aluminium, since Czapek (*12, p. 855*) reports that, as a rule, a content of more than 0.5 per cent of the total pure ash is not found. Here the aluminium makes up about 3.3 per cent of the total ash in the normal tops and nearly 4 per cent in the blighted tops. The proportion of aluminium to total ash is less in the roots than in the tops in health and in disease, one case excepted.

Wolff (*49*) reports spinach to have an average iron content of 0.552 per cent, calculated on the dry weight of the plant material, and with even this small quantity it is much richer in this element than the vast majority of plants that had then been studied.

It is interesting to note that healthy spinach tops were here found to contain an average of 0.132 per cent of iron, calculated on the dry weight, against 0.095 per cent in the blighted tops, in either case a quantity much larger than that recorded by Wolff. The roots of the diseased plants were found to contain an average of 0.122 per cent of iron and the roots of the normal plants 0.065 per cent. It seems as though a part of the iron that entered the plant through the roots accumulated there instead of going up to the leaves, as in the normal plants. It is interesting to note also that when calculated as percentage of the total ash the iron content of the roots always exceeds that of the corresponding tops, a relation more in evidence in diseased than in healthy roots.

OXIDASE REACTION IN HEALTHY AND IN BLIGHTED SPINACH

By H. H. Bunzell, *Formerly Chemical Biologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

A few years ago it was observed by Hasselbring and Alsberg (*18*) that spinach grown in the market gardens near Norfolk, Va., and affected by a disease resembling the mosaic of tobacco, had a greater oxidase content than healthy spinach from the same region. This observation coincided with the work by Woods (*50*, *51*) on the mosaic disease of tobacco. Since that time the writer has developed a quantitative method for the determination of oxidases, utilizing atmospheric oxygen (*7*). It was decided therefore to extend the observations of Hasselbring and Alsberg by comparing the oxidase activity of the leaves and roots of the diseased plants with those of healthy plants.

Three different collections of samples were made, designated as Set I, II, and III, respectively. In each case typically diseased plants were selected, as well as healthy control plants grown in the immediate vicinity of the diseased spinach. In all instances, therefore, the healthy and the diseased samples of the same set were grown under the same climatic and soil conditions. The plants were carefully washed in the laboratory to remove any adhering soil, and the surface water was removed by blotting the plants between sheets of filter paper. The leaves, freed from petioles and midribs, were dried over lime in a vacuum at room temperature. The roots were cut into pieces 2 to 3 mm. long and dried in a similar way. The samples were dried to constant weight and then powdered until the whole of the sample passed through a sieve of 100 meshes to the inch, after which they were kept in a desiccator.

The experiments were carried out according to the method formerly described (*10*). The following reagents were used: Pyrogallol, pyrocatechol, hydroquinone, phloroglucin, guaiacol, tyrosin, meta-cresol, paracresol, eugenol, and isoeugenol. The temperature at which the experiments were carried out was 37.4° C., and the rate of shaking five complete excursions in 3.4 seconds.

In most of the experiments the quantity of dry material used was so chosen as to give a reading of about 2 cm. In none of the experiments, however, was more than 0.10 gm. of the powder used, so that the readings were considerably below 2 cm. in the slightly active or inactive preparations. To make the results comparable, they were all calculated on the basis of 0.10 gm. of powder. The reagents were used in quantities ranging from 0.01 to 0.02 gm., this being an excess of the reagent in all cases. The results obtained are given in Table I.

TABLE I.—*Relative oxygen absorption of various oxidase reagents in the presence of healthy and diseased spinach material*

Material.	Pyrogallol.	Pyrocatechol.	Hydroquinone.	Phloroglucin.	Guaiacol.	Tyrosin.	Metacresol.	Paracresol.	Eugenol.	Isoeugenol.
SET I.										
Leaves:										
Normal.....	0.70	3.20	0.20	0.02	0.00	0.00	0.05	0.85	0.00	0.00
Pathological..	1.20	4.55	.52	.25	.10	.25	.27	.88	.00	.00
Roots:										
Normal.....	2.32	4.00	9.00	3.50	4.23	6.70	2.00	18.20	.00	.00
Pathological..	2.60	5.20	8.20	3.03	4.25	8.00	5.23	28.60	.00	.00
SET II.										
Leaves:										
Normal.....	1.40	5.40	.20	.00	.00	.00	.00	1.75	.00	.00
Pathological..	1.50	5.55	.55	.50	.32	1.00	.92	5.27	.02	.20
Roots:										
Normal.....	2.25	4.68	8.13	3.20	2.43	6.40	3.13	26.50	.07	.00
Pathological..	2.60	6.00	9.87	3.10	3.50	8.40	5.20	31.50	.10	.17
SET III.										
Leaves:										
Normal.....	.90	1.80	.33	.00	.00	.00	.00	.72	.00	.00
Pathological..	1.27	5.90	.23	.00	.00	.05	.07	1.52	.00	.00
Roots:										
Normal.....	1.15	1.90	4.00	2.20	1.50	4.60	2.63	20.70	.00	.00
Pathological..	2.27	2.80	7.00	3.60	2.20	8.20	2.40	31.50	.00	.10

Inasmuch as the various samples had different moisture contents, the results given in Table I are not strictly comparable. They were made so, however, by means of a calculation based on the following reasoning: If it be assumed that the total oxidase activity of the plant material is in the juice, and the weight of the solids dissolved in the juice be neglected, the oxidizing power of 0.10 gm. of juice can be calculated from the equation

$$a\ (\text{juice}) = a\ (\text{solids})\ \frac{\text{percentage of solids}}{\text{percentage of juice}}$$

The oxidizing power of 1 liter of juice is then necessarily 10,000 times the figures obtained in this way. But our unit of activity is the juice, 1 liter of which will transfer 8 gm. of oxygen (*8*), corresponding at 37.4° C. and 76 cm. pressure to 6,367 cc. The volume of the gas in the apparatus was 19 cc. The equation for calculating A, or the activity of the juice present in the fresh leaves and roots, is therefore

$$A = \frac{10{,}000}{25{,}475}\ a\ (\text{solids})\ \frac{\text{Percentage of solids}}{100 - \text{Percentage of solids}}$$

$$A = 0.393\ a\ (\text{solids})\ \frac{\text{Percentage of solids}}{100 - \text{Percentage of solids}}$$

The data which were presented in Table I are given in Table II, recalculated on the basis just described.

TABLE II.—*Oxidase activities of healthy and diseased spinach material*

[Results expressed in units]

Material.	Pyrogallol.	Pyrocatechol.	Hydroquinone.	Phloroglucin.	Guaiacol.	Tyrosin.	M-cresol.	P-cresol.	Eugenol.	Isoeugenol.
SET II.										
Leaves:										
Normal	0.087	0.337	0.019	0.000	0.000	0.000	0.000	0.109	0.000	0.000
Pathological	.102	.379	.038	.034	.022	.069	.063	.359	.001	.014
Roots:										
Normal	.178	.371	.645	.254	.193	.508	.248	2.101	.006	.000
Pathological	.278	.642	1.056	.332	.374	.899	.556	3.370	.011	.018
SET III.										
Leaves:										
Normal	.073	.147	.027	.000	.000	.000	.000	.059	.000	.000
Pathological	.139	.598	.023	.000	.000	.005	.007	.154	.000	.000
Roots:										
Normal	.154	.254	.535	.294	.201	.615	.352	2.768	.000	.000
Pathological	.280	.346	.864	.444	.271	1.024	.420	3.887	.000	.012

Table I shows three instances and Table II only two in which the activity of the healthy leaves or roots was greater than that of the diseased material. In all other cases there was either no measurable activity in both types of material or the diseased material was more active than the corresponding healthy material. This difference was from 50 to 100 per cent.

The figures expressing the activity of phloroglucinase, guaiacolase, tyrosinase, and meta-cresolase, are particularly interesting. These figures seem to indicate qualitative differences.

These results resemble those obtained in several other plant diseases. In the case of the mosaic of tobacco (*51*), the leafcurl of potatoes (*13*), the curly-top of sugar beets (*8*), and the curly-dwarf of potatoes (*9*), the diseased material shows a greater power to transfer atmospheric oxygen to certain aromatic compounds than the healthy material. In all these diseases the most characteristic symptom is a marked stunting of the plant. The following generalization seems therefore justified: In the above-mentioned plant diseases, which cause dwarfing of the plants affected, the capacity of the plant juice to utilize atmospheric oxygen for the oxidation of certain chromogens is abnormally increased. How this increase in the catalytic activity of the cell sap is brought about remains a problem. Whether the peroxid-forming substances are increased so that there is an increase in the oxygenases, which, in the presence of an excess of peroxidases might lead to the results outlined (*3*), or whether a greater quantity of specific activators are formed, which,

combined with various metabolic products form very unstable and readily oxidizable compounds, we are of course as yet unable to judge (*30*). It is possible that the difference observed was primarily of physical origin. Traube (*48*), in his paper on catalysis, recognized surface tension as one of the most important factors in the acceleration of biochemical reactions. The oxidase activity observed might be due simply to an increased concentration of the oxidizable material or oxygen, or both, in the layers adjacent to certain colloidal particles.

It is certain that in the course of stunting of growth there is an increase in the effectiveness of the oxidase mechanism. Whether this is the cause or a symptom of the disease is an open question. If we assume with Palladin (*37*) that with the aid of the respiratory pigments the oxidases are capable of carrying on the process of cell respiration, then it can readily be seen how an increased oxidation of some of the intermediate products of metabolism might seriously alter the course of the latter. Such plants could be said to be in a state of "fever" (*8*). If, on the other hand, oxidases are merely protective agents, as Portier (*38*) assumes, then the increased oxidase activity of the diseased plants would have to be ascribed to an attempt on the part of the plant to rid itself of poisonous products formed in the course of its abnormal metabolism.

CARBOHYDRATE PRODUCTION IN HEALTHY AND IN BLIGHTED SPINACH

By RODNEY H. TRUE, *Physiologist in Charge*, and LON A. HAWKINS, *Physiologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

Spinach plants affected with the blight show many symptoms pointing to a derangement of functions concerned with the carbohydrates, among which are the yellowish-green color and the sweetish taste. It was therefore deemed important to investigate these constituents in normal and in diseased plants. The material was taken from commercial fields near the Virginia Truck Experiment Station. The plants were carefully dug with a spade, and the soil adhering to the roots was quickly washed off. To reduce the translocation of products, the plants were divided into roots and tops, and after being loosely packed in market baskets were covered with paper and stored in a building in diffused light. The collection took place late in the forenoon on February 5, 1915, a clear day.

The samples were kept in a cool place while in transit and were taken to the laboratory at 7. 30 the next morning, where they were given immediate attention.

Starch, sucrose, and reducing sugars were determined by the usual methods. The results of these determinations are presented in Table I as percentages calculated on both fresh and dry weights of tops and on the fresh weights of the roots.

It will be noted that the samples of diseased tops have a somewhat greater percentage of dry matter than the healthy tops.

The reducing sugars under the conditions here given are clearly less abundant in the tops of blighted plants than in the normal samples, while in the roots but a trace is present in either type of material. The situation with reference to the sucrose in the tops, however, is quite the reverse, the diseased plants containing a considerably greater quantity than those in health. This difference is so great as to give a much higher total sugar content for the pathological material, a fact which in part accounts for the strikingly sweet taste found in the latter plants. It is of interest, however, to note that no noticeable sweetness is found in the normal leaves, although in the material collected in 1915 they contain nearly 80 per cent as much total sugars and nearly 65 per cent as much sucrose. Since the taste of sweetness is interfered with by a variety of other taste sensations, it is possible that certain substances having a marked taste may be present in the normal material and absent in that affected by the disease. On tasting the fresh material in the field it seemed that the characteristic "spinach" taste so strongly marked in the healthy leaves was almost lacking in the sweet diseased leaves.

TABLE I.—*Carbohydrates in healthy and in blighted spinach*

Carbohydrate.	Spinach tops.								Spinach roots, 1915.	
	Wet weight.				Dry weight.					
	Normal		Diseased.		Normal.		Diseased.		Wet weight.	
	1915	1916	1915	1916	1915	1916	1915	1916	Normal.	Diseased.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Dry weights of material.	.....	12. 53	.....	17. 43	.....	.....	.....	.....	.....	.....
	.....	12. 87	.....	16. 96	.....	.....	.....	.....	.....	.....
	11. 90	13. 42	11. 90	16. 10	.....	.....	.....	.....	.....	.....
	11. 50	13. 95	12. 50	18. 56	.....	.....	.....	.....	.....	.....
Average.....	11. 70	13. 19	12. 20	17. 26	.....	.....	.....	.....	.....	.....
Reducing sugars.....	. 49	. 28	. 52	. 26	4. 10	2. 23	4. 26	1. 49	.....	.....
	. 70	. 26	. 47	. 29	5. 98	2. 01	3. 85	1. 71	Trace.	Trace.
	. 69	. 25	. 45	. 26	5. 89	1. 86	3. 68	1. 61	.....	.....
	. 49	. 22	. 44	. 24	4. 10	1. 58	3. 60	1. 29	.....	.....
Average.....	. 59	. 25	. 47	. 26	5. 02	1. 89	3. 85	1. 50	.....	.....
Total sugars.....	1. 46	2. 48	2. 08	4. 77	12. 47	19. 79	17. 04	27. 36	.....	.....
	1. 36	1. 88	2. 18	5. 22	11. 62	14. 61	17. 86	30. 37	4. 07	6. 42
	1. 16	1. 60	1. 89	5. 49	9. 91	11. 92	15. 49	34. 10	5. 94	4. 17
	2. 03	2. 33	1. 83	4. 74	17. 36	16. 70	15. 00	25. 53	.....	.....
Average.....	1. 50	2. 07	1. 99	5. 05	12. 84	15. 69	16. 35	29. 26	5. 00	5. 30
Sucrose calculated by difference.....	. 93	2. 20	1. 50	4. 51	7. 94	17. 56	12. 29	25. 87	.....	.....
	. 63	1. 62	1. 64	4. 93	5. 44	12. 60	13. 44	29. 07	.....	.....
	. 45	1. 35	1. 38	5. 23	3. 84	10. 06	11. 31	32. 49	.....	.....
	1. 47	2. 11	1. 33	4. 50	12. 56	15. 12	10. 90	24. 24	.....	.....
Average.....	. 88	1. 82	1. 46	4. 79	7. 44	13. 80	11. 98	27. 76	.....	.....
Starch.....	. 95	1. 06	1. 53	1. 72	8. 11	8. 45	12. 54	9. 87	.....	.....
	. 79	1. 13	1. 51	1. 93	6. 75	8. 78	12. 37	11. 38	2. 03	2. 26
	. 73	. 99	1. 48	1. 57	6. 24	7. 38	12. 13	9. 75	2. 51	2. 58
	. 88	. 99	1. 51	1. 98	7. 52	7. 10	12. 37	10. 67	.....	.....
Average.....	. 84	1. 04	1. 51	1. 80	7. 15	7. 88	12. 35	10. 43	2. 27	2. 42

The starch content of the diseased tops is somewhat more than double that of the normal material. In the roots, both total sugars and starch were practically alike in both types of material.

From these results it appears justifiable to conclude that the cause of injury does not affect the machinery of photosynthesis or the materials used in carbohydrate manufacture to such an extent as to stop production. That this is carried on with equal efficiency in all parts, or with normal efficiency even in any part of the leaf, however, can not be stated. Indeed, the yellowish-green color representing an apparent reduction of chlorophyll would seem likely to go with a decreased activity in the

photosynthetic function. This condition recalls that of tobacco leaves when "mature" for cutting. The color changes to a more yellowish green, the leaves take on the brittle character seen in the diseased spinach and like it become gorged with starch.

It would hardly be safe to assume that photosynthetic activity is not impaired in the blighted plants in spite of the accumulation of carbohydrates. It is quite possible that impairment may be the case and that accumulation results from some interference with carbohydrate utilization.

In view of the destructive action of oxidases on diastatic enzyms, reported by Woods (*51*) in the case of tobacco mosaic, it was thought possible that here a somewhat similar situation was present. Since Bunzell in his investigation on this subject found the oxidase reaction with most reagents to be somewhat more intense in the diseased material, both leaves and roots, than in normal samples, it was thought necessary to determine the comparative diastatic activity of juices from these two types of material. The fresh leaves, after being ground in a mortar, were placed in a flask having a volume of 250 cc. and digested for 24 hours with 100 cc. of glycerin in an ice box at a temperature of about 10° C. This was then made up to volume, strained, and 50 cc. of the solution was added to 25 cc. of 1 per cent soluble starch. Controls were made in the same way from each sample to which no starch paste was added. One cc. of toluol was added to each flask to prevent the action of microorganisms. All preparations were allowed to stand at 30° C. for 48 hours, after which they were removed, cleared with lead acetate, made up to 100 cc. and filtered. The data given in Table II show the quantity of reducing sugar present in the preparations containing starch paste in excess of the controls from the same samples.

TABLE II.—*Diastatic activity in normal and in blighted spinach*

Date of collection.	Quantity used.	Glucose.	
		Normal leaves.	Blighted leaves.
	Gm.	*Gm.*	*Gm.*
February, 1915	100	0.316	0.308
Do	100	.304	.312
Do	100	.340	[a] .528
Average		.320	.310
March, 1916	50	.0130	.0142
Do	50	.0167	.0155
Do	50	.0172	.0121
Average		.0117	.0106

[a] Extracted with larger volume and calculated to the same basis as the others.

These results seem to point to the absence of any marked difference in the starch-digesting capabilities of normal and blighted spinach. This being the case it would seem to be indicated that the cause of carbohydrate accumulation should be sought in the deeper-lying metabolic processes in connection with which carbohydrates are utilized.

To recapitulate, it appears that in spinach-blight the process of carbohydrate manufacture is not inhibited, although it may be retarded. The reducing sugars are practically absent from the roots of all plants, while in the tops the normal plants contain somewhat more than the diseased. Both sucrose and starch are present in the leaves of the blighted plants in markedly greater quantity than in those of the normal plants. They are found in the roots of both healthy and diseased plants in approximately like quantities.

Determinations of diastatic activity failed to bring out any marked difference between healthy and diseased plants.

It is indicated that carbohydrate accumulation is due not to a breakdown of digestion but to some partial failure in the subsequent metabolic processes in connection with which carbohydrates are used.

NITROGEN METABOLISM IN NORMAL AND IN BLIGHTED SPINACH

By SAMUEL L. JODIDI, *Organic Chemist*, EDWARD H. KELLOGG, *Scientific Assistant*, and RODNEY H. TRUE, *Physiologist in Charge, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry.*

INTRODUCTION

In view of the evident derangement of the functions of nutrition in spinach-blight, it seemed necessary to undertake an investigation of the more fundamental processes of synthetic metabolism in the hope of learning in what way the disease causes injury. The purpose of this paper is to present the results gained from a study of the nitrogen metabolism.

Owing to their great importance in plant metabolism, certain groups of nitrogen-containing compounds demanded attention. The total nitrogen, the polypeptids and the proteins, closely connected as they seem to be with the fundamental activities of life in both plants and animals, the nature and quantity of the nonproteins present, especially of the amino acids, were studied in both healthy and diseased material.

For a number of years it has not been unusual in plant and soil investigations to estimate the protein content of biological materials by determining their nitrogen content (usually by the Kjeldahl method) and multiplying the nitrogen found by the factor 6.25. The literature contains a great number of such protein estimations. Evidently the investigators maintained that the nitrogen present in plant and soil materials is made up solely or chiefly of proteins or protein-like bodies, whose nitrogen percentage does not materially differ from that of proteins. Schulze and his coworkers (*41–45*) were among the first to demonstrate that such is not the case. They have shown, for instance, that a considerable portion of the nitrogen contained in Irish potatoes (*Solanum tuberosum*) and sugar beets, sometimes more than one-half, is made up of nonproteins (acid amids, amino acids, etc.). So far as the writers are aware, no similar systematic investigation concerning the character of the nitrogenous compounds in spinach has been made. Hence, it seemed of considerable importance to find out the nitrogen distribution in the spinach under normal physiological conditions. Futhermore, it was of interest to learn what changes, if any, had taken place in the nitrogen compounds of the spinach under pathological conditions.

Of special interest is the nature of the nonproteins (acid amids, amino acids, polypeptids) occurring in spinach. These compounds may be considered either as products of regressive metabolism in plants, or as products of synthesis in the latter built up out of inorganic nitrogen plant food, and are significant because of their food value, and the func-

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tions which they perform. Not only do they represent protein-saving materials, since in their presence the animal organism needs less protein for the maintenance of nitrogen equilibrium, but, a fact of still greater interest, recent investigations have demonstrated that animals can be maintained in nitrogen equilibrium, or even gain weight, when they are offered completely digested protein or amino acids instead of unchanged protein. This was shown to hold good for the organism of the dog (*1*), and, generally speaking, undoubtedly holds true for the animal organism. In this connection, it may not be amiss to mention that the various amino acids perform special functions in the body. Thus, the amino acids lysin and cystin have been recognized by Abderhalden (*1*), Osborne and Mendel (*32*, *33*, *34*, *35*, *36*) as necessary for the function of growth, and in this capacity can not be replaced by any other amino acids. Equally, the diamino acids histidin and arginin (*1*, *2*) are indispensable, since when they are removed from a complete amino acid mixture (obtained by protein hydrolysis), the remaining amino acids can no longer maintain the body in nitrogen equilibrium. Glycocoll (*1*), on the other hand, is not a necessary amino acid for the reason that it can be built up synthetically in the animal organism.

EXPERIMENTAL WORK

The spinach materials used in this investigation were secured in part from farms near Norfolk, Va.[1] Since some of these spinach materials (which were well mixed and treated as one lot) were not quite free from aphids, it was thought advisable to obtain samples free from the insects. These were taken from beds on another farm on May 6, 1916.[2] Three kinds of samples of the diseased and healthy plants, respectively, were prepared—namely, samples of the entire plant, of the tops, and of the roots. The samples were first fumigated with a tobacco preparation, then partly dried in the greenhouse for about a week, and partly in the electric drying oven at 40° to 50° C. The dried materials were ground, passed through a 40-mesh sieve, and kept in covered jars. As it was very soon discovered that the moisture content of the materials was changing, and also as it seemed more convenient to make practically all of the experiments with air-dried spinach, care was taken to make the ground spinach materials thoroughly air-dry. For this purpose they were exposed to the air in thin layers, in a place free from dust, until their moisture content became practically constant. The materials so prepared were kept in sealed jars ready for use. The results pertaining to the moisture content of the various spinach samples are expressed in Table I.

[1] By Dr. Rodney H. True, Dec. 1, 1915, and by the senior writer Jan. 21, 1916.
[2] By Mr. J. A. McClintock, then with the Virginia Truck Experiment Station.

TABLE I.—*Moisture content of air-dried healthy and diseased spinach*

HEALTHY SPINACH

No.	Date collected.	Kind of material.	Treatment of materials.	Quantity of air-dry substance used.	Water lost (at 100° C.). Observed.		Water lost (at 100° C.). Average.
				Gm.	*Gm.*	*Per cent.*	*Per cent.*
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant....	Dried in electric drying oven at 50° C. for 48 hours.	0.8944	0.0346	3.87	3.86
2	.....do.....	.....do.....	.....do.....	1.1323	.0436	3.85	
3	.....do.....	.....do.....	Remained in covered jars for about four weeks.	1.0000	.0437	4.37	4.31
4	.....do.....	.....do.....	.....do.....	1.0000	.0426	4.26	
5	.....do.....	.....do.....	Exposed to air in thin layers for 24 hours.	.7653	.0371	4.85	4.84
6	.....do.....	.....do.....	.....do.....	1.0338	.0499	4.83	
7	.....do.....	.....do.....	Exposed to air in thin layers for 24 hours longer.	.6197	.0316	5.10	5.10
8	.....do.....	.....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.9208	.0421	4.57	4.57
9	.....do.....	.....do.....	Remained in covered jars for about two weeks.	.8756	.0400	4.57	4.63
10	.....do.....	.....do.....	.....do.....	.9272	.0436	4.70	
11	.....do.....	Leaves.....	Dried in electric drying oven at 50° C. for 48 hours.	1.0000	.0372	3.72	3.78
12	.....do.....	.....do.....	.....	1.0000	.0383	3.83	
13	.....do.....	.....do.....	Remained in covered jars for about three weeks.	1.0000	.0416	4.16	4.17
14	.....do.....	.....do.....	.....do.....	1.0000	.0419	4.19	
15	.....do.....	.....do.....	Exposed to air in thin layers for 48 hours.	.9731	.0484	4.97	4.97
16	.....do.....	.....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.8422	.0381	4.52	4.52
17	.....do.....	.....do.....	Remained in covered jars about two weeks.	.7850	.0349	4.45	4.45
18	.....do.....	.....do.....	.....do.....	.9097	.0405	4.45	
19	.....do.....	Roots.....	Dried in electric drying oven at 50° C. for 48 hours.	1.0000	.0477	4.77	4.75
20	.....do.....	.....do.....	.....do.....	1.0000	.0473	4.73	
21	.....do.....	.....do.....	Remained in covered jars for about three weeks.	1.0000	.0529	5.29	5.29
22	.....do.....	.....do.....	.....do.....	1.0000	.0528	5.28	
23	.....do.....	.....do.....	Exposed to air in thin layers for 48 hours.	.6825	.0349	5.11	5.11
24	.....do.....	.....do.....	Exposed to air in thin layers another 24 hours (total, 72 hours).	.9110	.0456	5.01	5.01
25	.....do.....	.....do.....	Remained in covered jars for about two weeks.	.8498	.0433	5.10	5.00
26	.....do.....	.....do.....	.....do.....	.9164	.0448	4.89	
27	May 6, 1916....	Leaves.....	Exposed to air in thin layers for several days.	.9196	.0547	5.95	5.97
28	.....do.....	.....do.....	.....do.....	1.0508	.0623	5.93	
29	.....do.....	.....do.....	.....do.....	.6820	.0412	6.04	
30	.....do.....	Roots.....	.....do.....	.8288	.0593	7.15	7.12
31	.....do.....	.....do.....	.....do.....	.6087	.0431	7.08	

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant....	Dried in electric oven at 50° C.	1.0000	0.0415	4.15	4.18
2	.....do.....	.....do.....	.....do.....	1.0000	.0420	4.20	
3	.....do.....	.....do.....	Exposed to air in thin layers for 48 hours.	.8981	.0483	5.38	5.38
4	.....do.....	.....do.....	Exposed to air in thin layers for 24 more hours (total, 72 hours).	.8464	.0400	4.73	4.73
5	.....do.....	.....do.....	Remained in covered jars for about two weeks.	.8398	.0401	4.77	4.86
6	.....do.....	.....do.....	.....do.....	1.1441	.0568	4.96	
7	.....do.....	Leaves.....	Dried in electric oven at 50° C.	1.0000	.0582	5.82	5.81
8	.....do.....	.....do.....	.....do.....	1.0000	.0580	5.80	
9	.....do.....	.....do.....	Exposed to air in thin layers 48 hours.	1.2732	.0704	5.53	5.53

TABLE I.—*Moisture content of air-dried healthy and diseased spinach*—Continued.

DISEASED SPINACH—Continued.

No.	Date collected.	Kind of material.	Treatment of materials.	Quantity of air-dry substance used.	Water lost (at 100° C.).		
					Observed.		Average.
				Gm.	*Gm.*	*Per cent.*	*Per cent.*
10	Dec. 1, 1915, and Jan. 21, 1916.	Leaves	Exposed to air in thin layers 24 more hours (total, 72 hours).	0.9967	0.0517	5.19	5.19
11	do	do	Remained in covered jars for about two weeks.	.9315	.0502	5.39	5.48
12	do	do	do	1.0003	.0558	5.58	
13	do	Roots	Dried in electric oven at 50° C	1.0800	.0619	6.19	6.25
14	do	do	do	1.0000	.0630	6.30	
15	do	do	Exposed to air in thin layers for 48 hours.	.9147	.0464	5.07	5.07
16	do	do	Exposed to air in thin layers for 24 hours more (total, 72 hours).	.8112	.0445	5.49	5.49
17	do	do	Remained in covered jars for about two weeks.	.6920	.0410	5.92	5.93
18	do	do	do	.7512	.0447	5.95	
19	May 6, 1916	Leaves	Dried in electric oven at 40° C., which was followed by exposure of the substance to the air for several days.	.8313	.0532	6.40	6.27
20	do	do	do	.6923	.0426	6.15	
21	do	do	do	.9603	.0591	6.15	
22	do	do	do	.8570	.0545	6.36	
23	do	Roots	Treated like No. 19 to 22	.7860	.0533	6.78	6.76
24	do	do	do	.6100	.0411	6.74	

The examination of Table I reveals the fact that the drying of the spinach (entire plant) at 50° C. proceeded beyond the air-dry state, so that the moisture content of the material (which was kept in covered jars) increased from 3.86 to 4.31 per cent, and on exposure to the air in thin layers still further increased to 4.57 per cent. Further keeping of the material in covered jars showed that its moisture content remained practically constant, the small fluctuations being due undoubtedly to slight changes in the moisture of the air. The observations just mentioned hold good also for the spinach leaves and roots. The figures 4.63 per cent, the average for the entire plant, 4.45 per cent, average for the leaves, 5 per cent, average for the roots of the winter collection, and 5.97 and 7.12 per cent, respectively, for leaves and roots of the spring samples, were taken to represent the actual moisture percentages of the normal spinach materials in question, and were used in the calculation to the water-free basis of the results obtained in this investigation.

A glance at the second part of Table I shows that the samples of the entire plant of diseased spinach behaved very much like the healthy spinach, as far as the air drying is concerned. The diseased leaves and roots show a somewhat different behavior. Having been dried in the oven at 50° C. they still continued to decrease in their moisture content on exposure to the air in thin layers, which is evidently due to the fact that they had not been dried long enough in the oven. If not otherwise stated, the figures 4.86, 5.48, and 5.93 per cent for the whole plants, leaves, and roots, respectively, of the winter collection, and 6.27 and

6.76 per cent for the leaves and roots, respectively, of the spring collection, were used throughout this paper for calculating to the water-free basis the results obtained with the diseased materials in question

RELATION OF THE WATER CONTENT TO RETARDED GROWTH

The dwarfing effect of spinach-blight was shown in the weight of the plants used in a part of this work. Eighty diseased plants taken on the Jones farm weighed 552 gm., with an average weight of 6.9 gm. per plant. Forty-one healthy plants from the same beds weighed 647 gm., averaging 15.8 gm. per plant. The ratio of weights in disease and health was, therefore, 1 to 2.3. The leaves of the diseased plants were crisper, thicker in texture, and smaller in size than those of normally grown spinach. The root systems of the diseased plants were poorly developed, in comparison with those of the sound plants.

Considering the importance of water to the plant for the processes of transpiration, respiration, osmotic pressure, etc., it seemed worth while to make moisture estimations of the various plant tissues when in a fresh condition. Especially was it desirable to find out what difference, if any, there is between the healthy and the diseased spinach plants with regard to their moisture content. For this purpose several spinach samples were taken from various beds, immediately placed in air-tight jars, and the moisture estimations made within 24 hours. The results obtained are presented in Table II.

TABLE II.—*Moisture content of fresh healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Substance used.	Water lost (at 100° C.).		
				Observed.		Average.
			Gm.	*Gm.*	*Per cent.*	*Per cent.*
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant...	8.5906	7.2479	84.37	85.16
2	.....do.....	.....do.....	15.2711	13.1254	85.95	
3	.....do.....	Leaves.....	7.6908	6.7502	87.77	86.28
4	.....do.....	.....do.....	7.7867	6.6009	84.78	
5	May 6, 1916.....	Entire plant....	12.7295	10.6611	83.75	83.75
6	.....do.....	Leaves.....	11.5497	9.6796	83.81	83.81
7	.....do.....	Roots.....	2.1620	1.6991	78.59	78.59

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant...	7.5448	6.1100	80.98	81.55
2	.....do.....	.....do.....	5.8979	4.8691	82.56	
3	.....do.....	.....do.....	28.4032	23.0374	81.11	
4	.....do.....	Leaves.....	3.4600	2.9186	84.35	83.92
5	.....do.....	.....do.....	5.4050	4.5122	82.48	
6	May 6, 1916.....	Entire plant....	8.0617	6.4405	79.89	79.89
7	.....do.....	Leaves.....	7.4214	5.7435	77.39	77.39
8	.....do.....	Roots.....	1.1746	0.8963	76.31	76.31

When the two parts of the table are compared, it is readily seen that the healthy spinach samples, without exception, show a higher moisture content than the corresponding diseased samples. This is true not only of the entire plant, but of the leaves and roots as well. In other words, the spinach disease, physiologically characterized by a pronounced retardation of growth, is characterized chemically by a lower moisture content of its tissues. These data stand in full agreement with observations on certain animal tissues in disease and health. The water percentage of mouse carcinomata has been found by Cramer (*11*) to correspond with their rate of growth, the more rapidly growing tissues of the cancer showing a higher water content than the normal tissue, and vice versa. Evidently the rapidly growing plant cells like those of the cancer build up tissues with a comparatively high water content.

SPINACH NITROGEN

Since the main object was to ascertain the difference in quality and quantity of the nitrogenous compounds occurring in healthy spinach, on the one hand, and in diseased spinach, on the other, a number of nitrogen estimations were made according to the Gunning modification of Kjeldahl's method. As the moisture content of the spinach materials was not uniformly maintained it was necessary, in addition to nitrogen, also to run moisture estimations. The data which are recorded in Table III represent as a rule the average of two or more individual analyses.

TABLE III.—*Nitrogen content of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Percentage of nitrogen found in oven-dried substance.					
1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant.	Feb. 4, 4.73	Mar. 2, 4.66	Mar. 14, 4.76	Mar. 16, 4.84	Mar. 17, 4.90	Mar. 28, 4.79
2	.....do.....	Leaves....	Feb. 12, 5.08	Mar. 2, 4.93	Mar. 16, 5.03	Mar. 17, 5.02	Mar. 28, 5.07	.....
3	.....do.....	Roots.....	Feb. 12, 3.80	Mar. 2, 3.72	Mar. 17, 3.82	Mar. 28, 3.88	.....	.....
4	May 6, 1916 .	Leaves....	May 13, 3.39	May 15, 3.40	.....	.....	.....	.....
5	.....do.....	Roots.....	May 15, 2.36	.....	.....	.....	.....	.....

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916.	Entire plant.	Feb. 14–15, 3.59.	Feb. 17, 3.66	Mar. 15, 3.57	Mar. 16, 3.57	Mar. 17, 3.53	Mar. 29, 3.54
2	.....do.....	Leaves....	Feb. 14–15, 4.21.	Feb. 19, 4.13	Mar. 16, 4.31	Mar. 17, 4.31	Mar. 29, 4.22	.....
3	May 6, 1916	...do.....	May 13–15, 3.07.	.....	.....	.....	.....	.....
4	.....do.....	Roots.....	May 15, 2.74	.....	.....	.....	.....	.....

The examination of Table III (first part) reveals the fact that in the healthy plant the leaves have the highest and the roots the lowest nitrogen content, the figures for the entire plant lying between these two values. Likewise, by referring to Table III (second section) it may be

seen that in the diseased plant the nitrogen of the leaves is higher than that of either the roots or of the entire plant. A comparison of both sections further shows that the percentage of nitrogen in the healthy spinach (entire plant as well as leaves) is higher than in the corresponding diseased tissues, but that the nitrogen of the diseased roots by way of exception is somewhat higher than that of the healthy roots. If, as Boncquet (*6*) has claimed, denitrification sometimes takes place in diseased plant tissues whereby the nitrates are converted into nitrites and ammonia, the possibility of the ammonia escaping as such is not altogether out of the question. Such a proceeding would account for the smaller percentage of nitrogen in the diseased spinach.

PROTEIN NITROGEN OF THE SPINACH

The significance of protein as an integral constituent of protoplasm made it desirable to run a number of protein estimations of various healthy and diseased spinach tissues. The method applied here was originally proposed by Ritthausen (*39*) and perfected by Stutzer (*47*). One gm. of the finely powdered air-dry material was treated in a beaker with 100 cc. of water, heated to boiling, and kept on the steam bath for about 10 minutes. About 2 cc. of a concentrated potassium-alum solution were added, followed by 15 cc. of Stutzer's solution (corresponding to 0.45 gm. of copper hydroxid), and the whole was well stirred. On cooling, the insoluble residue was filtered off, washed with water, and the nitrogen estimated according to Kjeldahl's method. In a few instances, which will be mentioned, the following modification by Stutzer was used: To 1 gm. of the substance were added 100 cc. of absolute alcohol and 1 cc. of acetic acid, heated to the boiling point on a steam bath, and allowed to settle, when the supernatant liquid was carefully decanted through a filter. The substance which remained in the beaker was now treated with 100 cc. of water, heated to boiling, etc., as already outlined. The data obtained are summarized in Table IV.

While the modified method of Stutzer yields a somewhat higher percentage of protein nitrogen than the ordinary Stutzer method, it will be noticed by reference to the first section of Table IV that the proportion of protein nitrogen in leaves 8 to 10 is practically the same as in the roots 11 to 13. In other words, the protein nitrogen is practically uniform throughout the healthy spinach plant (see No. 1–7). As regards the actual quantities of protein nitrogen in the plants, as seen in the relation of protein nitrogen to dry weight, the leaves are considerably richer than the roots in both winter and spring samples. However, it will be noted that the spinach collected in May, 1916, shows a very much higher proportion of protein nitrogen than that collected in December, 1915, and January, 1916. The percentage of protein nitrogen calculated on dry weight is, however, greater in the plants of the earlier collection. The high relative percentage of protein nitrogen may perhaps be due to the fact that the winter samples were not as mature as those collected in May.

TABLE IV.—*Protein nitrogen of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Date when estimation was made.	Protein nitrogen found.		
				Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen.
1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Mar. 13	0.356	2.40	51.56
2	do	do	do	.361	2.43	52.25
3	do	do	Apr. 1	.347	2.34	48.80
4	do	do	do	.344	2.32	48.42
	Average (1–4)			.352	2.37	50.26
5[a]	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Mar. 11	.370	2.49	53.44
6[a]	do	do	do	.365	2.46	52.79
7[a]	do	do	Apr. 8	.353	2.38	49.72
	Average (5–7)			.363	2.44	51.98
8	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 1	.350	2.55	50.21
9	do	do		.346	2.52	49.77
	Average (8–9)			.348	2.54	49.99
10[a]	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 8	.362	2.63	51.79
11[a]	do	Roots	Apr. 1		1.93	49.84
12[a]	do	do	do		1.89	48.75
	Average (11–12)				1.91	49.30
13[a]	Dec. 1, 1915, and Jan. 21, 1916	Roots	Apr. 8		2.04	52.50
14[a]	May 6, 1916	Leaves	May 17	.343	2.12	62.50
15[a]	do	do	do	.345	2.13	62.65
	Average (14–15)			.344	2.13	62.58
16	May 6, 1916	Roots	May 19	.319	1.49	63.28
17	do	do	do	.323	1.51	63.96
	Average (16–17)			.321	1.50	63.62

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Apr. 3	0.376	2.04	57.37
2	do	do	do	.371	2.01	56.83
	Average (1–2)			.374	2.03	57.20
3[a]	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	Apr. 8	.384	[illegible].08	58.70
4	do	Leaves	Apr. 3	.376	2.34	55.53
5	do	do	do	.370	2.30	54.45
	Average (4–5)			.373	2.32	54.99
6[a]	Dec. 1, 1915, and Jan. 21, 1916	Leaves	Apr. 8	.388	2.41	57.08
7	do	Roots	Apr. 3		2.54	61.38
8	do	do	do		2.50	60.37
	Average (7–8)				2.52	60.87
9[a]	Dec. 1, 1915, and Jan. 21, 1916	Roots	Apr. 8		2.52	60.84
10	May 6, 1916	Leaves	May 17	.427	1.89	61.43
11	do	do	do	.414	1.83	59.45
	Average (10–11)			.421	1.86	60.44
12	May 6, 1916	Roots	May 19	.450	1.90	69.3
13	do	do	do	.450	1.90	69.2
	Average (12–13)			.450	1.90	69.2

[a] Modified Stutzer's method.

Table IV, second part, shows that the protein nitrogen when referred to the total nitrogen in the diseased spinach is not distributed uniformly, the percentage in the roots being higher than that in the leaves. Here the samples collected in December and January have a considerably lower proportion of protein nitrogen referred to total nitrogen than the sample gathered in May. When referred to the dry weight of the plant material, the protein nitrogen of the roots exceeds that in the leaves in both winter and spring collections, the quantity present in the spring samples being clearly less than in those taken in winter. In this latter respect the diseased plants differ from the normal ones. The greater proportion of protein nitrogen to total nitrogen may indicate that the spring sample was in a riper state than the former winter sample.

When the first section of Table IV isco mpared with the second section, it is seen that the diseased spinach is not only able to build up protein but, with one exception, shows even a higher percentage of protein nitrogen (calculated on total nitrogen) than the healthy spinach, this being true of the leaves, the roots, and the entire plant. In case the protein nitrogen is related to the dry weight of the plant tissues, the situation is reversed as regards the entire plant and the leaves, the roots only showing a higher content in the diseased plants. This is true for both winter and spring material. So far as animal tissues are concerned, it was shown by Cramer (*11*) that certain rapidly growing cells and tissues build up protoplasm with less complex organic compounds (like proteins, etc.) than more slowly growing tissues.

From the data here presented it would seem that in spinach collected both in winter and spring the actual number of grams of protein nitrogen is greater in a given dry weight of healthy tops than in a like quantity of diseased material, this relation being plainly reversed in the case of the roots. It also seems clear that of the total nitrogen content a greater percentage is in protein form in the diseased than in the normal plants, the case of the leaves in the spring material being the only exception. It is further indicated that all winter material, diseased and healthy, is somewhat richer in protein nitrogen than the corresponding material collected in the spring.

NONPROTEIN NITROGEN OF THE SPINACH

The nonprotein nitrogen is usually found by subtracting the protein nitrogen from the total nitrogen. It seemed, however, desirable to make direct estimations of the nonprotein nitrogen as a check on the protein determinations. We proceeded as follows: The combined filtrate and washings from the copper precipitate, as obtained in the protein estimation according to Stutzer's method, were usually acidulated, concentrated on the water bath, quantitatively transferred to a Kjeldahl flask, and the nitrogen estimated as usual according to the Gunning modification of Kjeldahl's method. The results obtained are reported in Table V.

TABLE V.—*Nonprotein nitrogen of healthy and diseased spinach*

HEALTHY SPINACH

No.	Date when spinach was collected.	Kind of material.	Date when estimation was made.	Nonprotein nitrogen found.			Nonprotein nitrogen calculated (difference from 100). Per cent of total nitrogen.
				Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen	
1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	March 13	0.280	1.89	40.5	48.44
2	do	do	do	.280	1.89	40.4	47.75
3	do	do	April 1	Lost.	.....	.....	51.20
4	do	do	do	.331	2.23	46.65	51.58
	Average (1–4)			.297	2.00	42.52	49.75
5	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	March 11	.288	1.94	41.69	46.56
6	do	do	do	.282	1.90	40.75	47.21
7	do	do	April 8	.303	2.04	42.69	50.28
	Average (5–7)			.291	1.96	41.71	48.02
8	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 1	.313	2.28	45.02	49.79
9	do	do	do	.303	2.21	43.58	50.23
	Average (8–9)			.308	2.25	44.30	50.01
10	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 8	.285	2.08	41.12	48.21
11	do	Roots	April 1	Lost.	.....	.....	50.16
12	do	do	do	.....	1.90	48.91	51.25
	Average (11–12)			.....	1.90	48.91	50.71
13	Dec. 1, 1915, and Jan. 21, 1916	Roots	April 8	.....	1.72	44.38	47.50
14	May 6, 1916	Leaves	May 17	.212	1.31	38.66	37.50
15	do	do	do	.198	1.22	35.81	37.35
	Average (14–15)			.205	1.27	37.24	37.43
16	May 6, 1916	Roots	May 19	.161	.75	31.75	36.72
17	do	do	do	.161	.75	31.75	36.04
	Average (16–17)			.161	.75	31.75	36.38

DISEASED SPINACH

1	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	April 3	0.286	1.55	43.76	42.43
2	do	do	do	.288	1.56	44.18	43.17
	Average (1–2)			.287	1.56	43.97	42.80
3	Dec. 1, 1915, and Jan. 21, 1916	Entire plant	April 8	.271	1.47	41.48	41.30
4	do	Leaves	April 3	.307	1.91	45.22	44.47
5	do	do	do	.294	1.83	43.27	45.55
	Average (4–5)			.301	1.87	44.25	45.01
6	Dec. 1, 1915, and Jan. 21, 1916	Leaves	April 8	.288	1.79	42.39	42.92
7	do	Roots	April 3	.....	1.64	39.65	38.62
8	do	do	do	.....	1.66	40.16	39.63
	Average (7–8)			.....	1.65	39.91	39.13
9	Dec. 1, 1915, and Jan. 21, 1916	Roots	April 8	.....	1.63	39.29	39.16
10	May 6, 1916	Leaves	May 17	.274	1.21	39.3	38.57
11	do	do	do	.274	1.21	39.3	40.55
	Average (10–11)			.274	1.21	39.3	39.56
12	May 6, 1916	Roots	May 19	.199	.84	30.49	30.7
13	do	do	do	.194	.82	29.98	30.8
	Average (12–13)			.197	.83	30.24	30.8

Examination of Table V, first section, shows that the calculated percentage of nonprotein nitrogen is usually higher than that found directly by analysis. This is especially marked in the case of No. 1 to 10, in which the filtrates from protein copper precipitate were concentrated on the water bath without having previously been acidulated. However, where acidulation of the filtrates did take place, as in No. 11 to 17, the difference in the results is still not inconsiderable. It seems reasonable to ascribe the loss of nonprotein nitrogen, at least in part, to the heating of the spinach with copper hydroxid (incidental to the Stutzer method) whereby the amids, which, as will be shown later, are contained in the spinach, undoubtedly lose a part of their nitrogen as ammonia in addition to the loss of ammonia present as such in the plant. From the data, which will be given later in this paper, it will be noticed that the sum of ammoniacal and acid amide nitrogen in the healthy spinach tissues is, as a rule, higher than the percentage present in the diseased tissues. For this reason it could be expected that the difference between the nonprotein nitrogen found and the nonprotein nitrogen calculated would be greater in the case of the healthy plant tissues. Just why in the case of the diseased spinach the nonprotein nitrogen calculated and found is practically the same needs still further investigation.

EXTRACTION OF THE NONPROTEIN NITROGEN OF THE SPINACH

A preliminary experiment was made to ascertain how the nitrogen can best be extracted from the spinach. Fifteen gm. of air-dry healthy spinach were treated in a round-bottom flask with 100 cc. of boiling hot ammonia-free water and digested on the steam bath with frequent shaking for 15 minutes, when the substance was filtered through a Buchner funnel provided with a filter, the extraction being repeated three more times. The final cake remaining on the Buchner funnel was thoroughly washed with boiling hot water. The combined filtrates and washings were acidified with acetic acid, boiled for a few minutes, filtered, and washed as usual on a filter. The filtrate and washings were cooled and made up to 1 liter. Two more portions of 15 gm. each were treated as outlined, with the difference that these two portions were extracted six and eight times, respectively. Nitrogen estimations in aliquots of the three extracts, showed that they contained, respectively, 55.86, 58.28, and 59.31 per cent of the total nitrogen. Inasmuch as the healthy spinach contains about 50 per cent of protein nitrogen (see Table IV), the fact that the three extracts contained nitrogen in excess of what could be expected, and more of it the more frequently the substance was extracted, seemed to indicate that a part of the protein nitrogen went into solution, probably through peptonization. The extraction was then modified so as to use a smaller amount of water and to effect the extraction more rapidly. The procedure was as follows:

Eighty gm. of air-dried spinach material were introduced into two 500 cc. round-bottom flasks (about equal amounts), and 200 cc. of boiling hot ammonia-free water added to each of the flasks, which were now kept on the water bath for 10 to 15 minutes. The digested spinach was then sucked off through a Buchner funnel, provided with a linen cloth filter (instead of a paper filter which filters very slowly). The cake remaining on the Buchner funnel was transferred to the round-bottom flasks, hot water added to original volume (about 150 cc. water to each flask), kept on the steam bath, etc., the extraction having been effected altogether four times. The combined extracts were distinctly acidified with acetic acid, using a small excess of it, and boiled for a few minutes. The extracts so treated were then filtered and washed on a Gooch crucible provided with a paper-pulp filter which, as was shown by Jodidi and Kellogg, proved to be an efficient filter not only for the estimation of phosphoric acid (*25*), calcium and magnesium (*26*), and in general for quantitative analysis (*27*), but also for the separation of solids from liquid (*28*) in general chemical work, especially when a comparatively small precipitate is contained in a large volume of liquid. On cooling, the liquid was made up to 2,000 cc., of which two or three portions of 25 cc. each were oxidized according to Kjeldahl's method to ascertain the amount of nitrogen extracted. The data are summarized in Table VI.

TABLE VI.—*Nitrogen in water extract of healthy and diseased spinach*

No.	Date when spinach was collected.	Kind of material.	Nitrogen found.			
			Per cent of fresh weight.	Per cent of oven-dry weight.	Per cent of total nitrogen.	Nitrogen in 25 cc. of extract.
						Mgm.
1	Dec. 1, 1915, and Jan. 21, 1916.	Healthy spinach (entire plant).	0.375	2.53	52.85	0.9656
2	.....do.....	Healthy leaves.....	.353	2.57	50.64	.9812
3	.....do.....	Diseased spinach (entire plant).	.343	1.86	52.40	.7060
4	.....do.....	Diseased leaves.....	.357	2.22	52.72	.8412
5	May 6, 1916.....	.....do.....	.352	1.56	50.74	.5840

A glance at Table VI shows that under the conditions outlined the proportions of nitrogen extracted by water from the various spinach materials were fairly uniform, this being true of both the healthy and the diseased plants. When we further compare No. 1 and 2 of Table VI with No. 1 and 13 of Table IV, first section, we find that the total sum of the water-soluble nitrogen and the protein nitrogen is from 1 to 2 per cent above 100, this being undoubtedly due to the fact that the Stutzer method ordinarily yields a somewhat too high percentage of protein nitrogen. The discrepancy is, however, greater in the case of the diseased

spinach, where the amounts of soluble nitrogen plus protein nitrogen range from 106 to 110 per cent. Giving due allowance for the inaccuracies of the operation involved, it is reasonable to ascribe the discrepancies noticed, not merely to the high results of Stutzer's protein method, but also to the possibility that the protein of the diseased spinach examined may perhaps differ from that of the healthy spinach in undergoing changes more readily.

DISTRIBUTION OF THE WATER-SOLUBLE NITROGEN IN SPINACH

The determination of the nitrogen of acid amids, diamino acids, and monoamino acids was made essentially according to Hausmann's method (*19*, *20*), as modified by Osborne and Harris (*31*), and as applied to soils by one of the writers (*Jodidi 21*, *22*, *23*, *24*). The estimation of the nitrogen of compounds other than those mentioned was made according to methods which will briefly be described subsequently in this paper.

Ordinarily to 250 cc. of spinach sclution, prepared as outlined above and corresponding to 10 gm. of air-dry spinach, concentrated hydrochloric acid was added to a concentration of 20 per cent, and boiled under a reflux condenser 30 minutes. The hydrolyzed substance was now quantitatively transferred to a porcelain dish and evaporated on the steam bath practically to dryness.

In order to ascertain whether or not all of the acid amid nitrogen was split off as ammonia under the conditions outlined, and at the same time to completely hydrolyze any polypeptids present, another portion of 250 cc. of the same spinach extract was treated with enough concentrated hydrochloric acid to give a 20 per cent concentration and boiled under a reflux condenser for 8 hours. The fact that not all of the nitrogenous constituents of plants are known, and the consideration that some of them might be decomposed by boiling with 20 per cent hydrochloric acid, made it desirable to hydrolize the spinach extract with as dilute an acid as possible, but strong enough to split off quantitatively in the form of ammonia the nitrogen of acid amids present. Inasmuch as asparagin and glutamin are probably the principal acid amids contained in plants, their behavior toward hydrochloric acid of different strength was here of special interest. Unfortunately, the writers had to confine the experiment to asparagin only, not having any glutamin. Its chemical behavior, however, is known to be very similar to that of asparagin, its lower homolog.

Two and five-tenths gm. of crystallized asparagin, with a nitrogen percentage of 18.38 (the formula $C_4H_8N_2O_3 + H_2O$ requires 18.66 per cent N), were dissolved in water and made up to 350 cc. Of this solution portions of 20 cc., each, were transferred to small round-bottom flasks to which concentrated hydrochloric acid was added until the desired per-

centage was obtained, and boiled under reflux for a definite time. No. 1 and 2 were then neutralized with sodium hydroxid, and on addition of 3 gm. of magnesium oxid were subjected to distillation, while No. 3 to 14 were directly distilled with 3 gm. of magnesia. The results are recorded in Table VII.

TABLE VII.—*Hydrolysis of asparagin with hydrochloric acid*

No.	Strength of hydrochloric acid.	Boiled under reflux.	Ammoniacal nitrogen found.	
			Observed.	Average.
	Per cent.	*Hours.*	*Mgm.*	*Per cent.*
1	20	½	12.48	.....
2	20	½	12.48	47.53
3	2	1	11.43	.....
4	2	1	11.50	43.68
5	3	1	Lost.	.....
6	3	1	11.61	44.22
7	4	1	12.34	.....
8	4	1	12.05	46.46
9	2	2	11.92	.....
10	2	2	12.22	45.97
11	3	2	12.27	.....
12	3	2	12.36	46.92
13	4	2	12.36	.....
14	4	2	12.44	47.22

From the table it follows that boiling asparagin with 4 per cent hydrochloric acid for 2 hours split off, in the form of ammonia, as much nitrogen as did boiling with 20 per cent hydrochloric acid for 30 minutes.

DESCRIPTION OF METHODS

(1) THE TOTAL SOLUBLE NITROGEN, as mentioned already, was usually estimated in 25-cc. portions of the aqueous spinach extract according to the Gunning modification of the Kjeldahl method.

(2) THE NITROGEN OF AMMONIA present as such in the spinach materials was estimated according to Grafe's method (*14*), which is based upon the work of Wurster, Boussignault, Folin, Krüger, and Reich. Ordinarily 20 gm. of air-dry spinach were introduced into a 2-liter round-bottom flask with the aid of 50 cc. of saturated sodium-chlorid solution, 50 cc. of distilled water, 25 cc. alcohol, and the whole mixed thoroughly. The flask was then connected with a Péligot tube (of about 400 cc. capacity) usually containing 20 cc. of *N*/5 sulphuric acid, whereupon 25 cc. of saturated sodium-carbonate solution were added. After the whole apparatus was carefully made air-tight, the burner under the water bath was lighted and the suction pump (May-Nelson) brought into action. The first 3 hours the distillation took place at 25° to 28° C., the last 3 or 4 hours at about 37°. The absolute pressure observed was mostly about 20 mm. (ranging from 5 to 45 mm.). The ammonia found in the spinach by this method was taken to represent also the ammonia in the aqueous spinach extract. A direct ammonia determination in the spinach extract, because of the heat applied at the extraction, was deemed inaccurate.

(3) THE ACID AMID NITROGEN was estimated in 250-cc. portions of the spinach extract. The latter, on hydrolysis, was evaporated to dryness, transferred quantitatively to an 800-cc. Kjeldahl flask of Pyrex glass with the aid of 100 cc. of distilled

water. Two grams of magnesium oxid, previously reduced to cream with 100 cc. of water, were added to the flask and distilled, the distillate being received in an Erlenmeyer flask containing *N/10* sulphuric acid. From the ammoniacal nitrogen found by titration the ammonia nitrogen found in (2) was subtracted, giving the nitrogen of the acid amids.

That the distillates obtained at the distillation of the hydrolyzed and evaporated spinach extracts with magnesium oxid actually represented ammonia was shown by the preparation of chloroplatinates from the distillates in question. The platinum double salts usually, though not always, showed a platinum percentage which was close to the 43.93 required by the formula $(NH_4)_2PtCl_6$.

(4) THE HUMIN NITROGEN, which resulted from the action of the boiling hydrochloric acid upon the aqueous spinach, and which was due in part to the presence in the latter of diamino acids and monoamino acids, was estimated in the magnesium-oxid residue remaining in the Kjeldahl flask from the acid amid estimation in (3). The magnesium-oxid residue was completely decanted off on a Gooch crucible provided with a linen cloth filter, the filtrate being received in a beaker placed in Witt's filtering apparatus. The residue was now repeatedly (about 10 times) treated with small quantities (25 cc.) of boiling hot water, and finally quantitatively filtered and washed on the Gooch crucible (filtrates and washings being received in another beaker). The magnesium-oxid cake with the aid of dilute sulphuric acid was then quantitatively transferred to a 500 cc. Kjeldahl flask and the nitrogen estimated according to Kjeldahl's method.

(5) THE BASIC NITROGEN was estimated in the filtrates and washings from the magnesium-oxid cake obtained in (4). The combined liquids, with the exception of the first decantation, which in order to avoid the formation of a brown precipitate was not concentrated, were evaporated on the water bath to a small volume. This was added to the first decantation, cooled to 20° C., made up to 100 cc., and treated with 5 gm. of sulphuric acid, 30 cc. of a solution containing 20 gm. of phosphotungstic acid, and 5 gm. of sulphuric acid per 100 cc. After at least 24 hours, the precipitate was filtered through an S. and S. filter and washed with about 200 cc. of a solution containing 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 cc., the washing being effected by rinsing the precipitate from the filter into a beaker and returning to the filter three times. The washed precipitate was then Kjeldahlized and tritrated, giving the proportion of the basic nitrogen.

That diamino acids formed a part of the basic nitrogen was demonstrated in the following way: Another portion of the phosphotungstic-acid precipitate obtained in the manner outlined was treated with barium hydroxid in excess, and the barium phosphotungstate filtered out and washed. Filtrate and washings were not treated with carbon dioxid to remove the excess of baryta, the filtrate and washings from barium carbonate being evaporated on the water bath to a small volume. This concentrated solution gave the following tests:

1. Phosphotungstic-acid solution gave immediately a heavy, white precipitate.
2. Phosphomolybdic acid gave a yellow precipitate.
3. Mercuric chlorid gave a gray flocculent precipitate.
4. Silver nitrate gave a grayish-white precipitate, soluble in excess of ammonia.
5. The solution was distinctly alkaline.
6. Addition of neutralized formaldehyde to the alkaline solution made it turn acid, pointing to the presence of carboxyl and amino groups.

(6) THE MONOAMINO ACID NITROGEN was estimated in the filtrate from the phosphotungstic-acid precipitate. To remove the excess of phosphotungstic and sulphuric acids from the filtrate the latter was treated with barium hydroxid whose excess was removed with carbon dioxid. Both the barium phosphotungstate (plus barium sulphate) and the barium-carbonate precipitates were repeatedly washed with boiling

hot water. Filtrate and washings from barium carbonate were evaporated on the water bath to small volume, filtered, washed, and finally made up to 100 cc., of which 20 cc. were Kjeldahlized to ascertain the quantity of nitrogen present. The remaining 80 cc. were formol-titrated, having previously been freed from carbon dioxid and phosphoric acid whose presence would interfere with formol titration (*46*). For this purpose there were added to the 80 cc. of the solution 2 gm. of barium chlorid which were dissolved by shaking, then 1 cc. of a 0.5 per cent of phenolphthalein solution and enough of saturated barium hydroxid solution until a red color appeared. Five cc. of *N/5* barium hydroxid were added in excess, made up to the mark with water (usually 100 cc.), shaken and filtered after a few minutes. Of this filtrate definite quantities, usually of 40 cc. each, were neutralized with *N/5* hydrochloric acid and formol titrated, the data of the titration being recalculated to the total soluble nitrogen. In cases in which the solution was too dark for formol titration, it was decolored by the formation in the solution of a precipitate of silver chlorid (or copper sulphid). Ordinarily the solution was rendered acid with *N/2* hydrochloric acid, whereupon about 10 cc. of *N/2* silver-nitrate solution was gradually added while the flask was constantly shaken. Inasmuch as the presence of silver would interfere with the formol tritration, care was taken to insure an excess of the chlorin ion in the solution by adding to it about 5 cc. of *2 N* barium-chlorid solution. The silver-chlorid precipitate formed in the solution usually carries down the coloring substance, so that the filtrate shows a yellowish light color and can then readily be formol titrated.

(7) THE PEPTID NITROGEN was estimated in the aqueous spinach extract, on hydrolysis with 20 per cent hydrochloric acid for 8 hours. From the hydrolyzed solution ammonia and humin nitrogen were removed in the manner already described. The filtrate and washings from magnesium-oxid residue were then evaporated on the water bath, cooled, and made up to 100 cc., of which 20 cc. were Kjeldahlized to ascertain the nitrogen present. The remaining 80 cc. were freed from carbon dioxid, phosphoric acid, and coloring matter, as outlined above. Aliquots of the filtrate (made up to 100 cc.) usually portions of 40 cc. each, were then formol-titrated. From the amino acid nitrogen found here, was subtracted the amino-acid nitrogen (minus the ammonia present as such) which was found directly in the water extract of the spinach materials by formol titration.

(8) THE RESIDUAL SOLUBLE NITROGEN made up of nitrogenous compounds other than those given above constitutes the difference between the total water-soluble nitrogen and the sum of the nitrogen found as ammoniacal nitrogen in (2), acid amid nitrogen in (3), humin nitrogen in (4), basic nitrogen in (5), monoamino acid nitrogen in (6), and peptid nitrogen in (7).

The results obtained by the methods described are summarized in Table VIII.

As will be seen, the first section of Table VIII presents the results expressed in percentage of the total soluble nitrogen of the spinach materials, while in the second and third sections the data are expressed in percentage of the total nitrogen and of the oven-dried spinach material, respectively.

The total nitrogen of the spinach is given in Table III. The examination of the latter part of this table shows that, with one exception,[1] the nitrogen content of all the healthy spinach materials is higher than the nitrogen content of the corresponding diseased materials. It is true that investigation showed that the soil of the diseased spinach has a

[1] Compare No. 5 of Table III, first part, with No. 4 of the second part.

somewhat higher concentration of salts than the soil of the healthy plants; other properties, however, like humus content, water-holding capacity, were in favor of soil poorer in nitrogen. If any clear difference existed, the soil of the diseased spinach was superior to that yielding the healthy spinach. This being true, it was evident that the cause of the lower nitrogen content of the diseased spinach was to be sought in the plant itself.

The loss of nitrogen may occur through denitrification, which, if it does take place in the diseased spinach, would satisfactorily explain its lower nitrogen content as well as other phenomena which will be mentioned subsequently in this paper. The first step in the process of denitrification consists primarily in the reduction of the nitrates to nitrites. The latter react on acid amids, which, as we have seen, are present in the spinach tissues, whereby elementary nitrogen is set free. This reaction can be presented chemically as follows:

$$\underbrace{R.CO.NH_2}_{\text{Acid amid.}}+NO.OH=\underbrace{R.CO.OH}_{\text{Fatty acid.}}+N_2+H_2O.$$

Thus, both the nitrogen of nitrates and of acid amids would be lost through the process of denitrification, explaining the lower nitrogen content in the diseased spinach.

In the process of denitrification the reduction seems not to stop after the nitrates have been reduced to nitrites, but the latter seem often in part to be further reduced to ammonia. If this took place, it is evident that the diseased spinach tissues would show a somewhat higher percentage of ammoniacal nitrogen than the corresponding healthy tissues. This is actually the case, as a glance at the data from the winter-collected material in Table VIII shows (column 7).

Conversely, a somewhat smaller acid amid nitrogen content would be expected in the diseased spinach tissues than in the corresponding healthy tissues, because of the fact that the acid amids by reacting with the nitrites in accordance with the supposed reaction would lose their nitrogen in gas form. Evidence to support this explanation is found in Table VIII (column 8).

An examination of column 9 of the same table shows that the percentage of humin nitrogen in the diseased spinach is, as a rule, higher than in the healthy spinach. Hart and Bentley (*15*) and Roxas (*40*) in Hart's laboratory have demonstrated that the formation of humin nitrogen takes place at the expense of diamino acids and monoamino acids. Inasmuch as, under similar conditions, the humin nitrogen was formed in the spinach extract by boiling with hydrochloric acid, it is evident that the proportions of monoamino acids and basic nitrogen originally present were higher than the values given in Table VIII (columns 10 and 11)—namely, by the amount of humin nitrogen formed

TABLE VIII.—*Distribution of the water-soluble nitrogen in spinach*

RESULTS EXPRESSED IN PERCENTAGE OF THE TOTAL SOLUBLE NITROGEN OF THE SPINACH MATERIALS

No.	Date when spinach was collected.	Spinach material.	Strength of hydrochloric acid.	Time of digest.	Total nitrogen (H_2O soluble).	Ammonia nitrogen.	Acid amid nitrogen.	Humin nitrogen.	Basic nitrogen.	Monoamino acid nitrogen.	Peptid nitrogen.	Residual soluble nitrogen.
			Per cent.	*Hours.*								
1	Dec. 1, 1915, and Jan. 21, 1916	Healthy, entire plant	4	2	100	6.09	13.25	5.73	25.95	18.74	.....	.....
2	do	do	20	½	100	6.09	16.32	6.46	24.44	20.61	.....	.....
3	do	do	20	8	100	6.09	22.19	4.67	.....	.....	4.66	17.34
4	do	Healthy leaves	20	8	100	4.82	22.66	5.41	.....	.....	7.48	10.56
5	do	do	20	11	100	4.82	23.92	5.01	.....	.....	.....	.....
6	do	do	20	½	100	4.82	17.77	5.23	30.12	18.95	.....	.....
7	do	do	4	2	100	4.82	14.21	7.51	29.84	19.40	.....	.....
8	do	do	20	½	100	4.82	18.38	7.17	27.42	19.13	.....	.....
9	do	do	4	2	100	4.82	13.63	6.42	28.06	18.09	.....	.....
10	do	Diseased, entire plant	4	2	100	8.14	13.53	10.53	27.47	18.83	.....	.....
11	do	do	20	½	100	8.14	14.77	7.06	27.79	22.07	.....	.....
12	do	do	20	8	100	8.14	17.56	5.76	.....	.....	3.21	15.47
13	do	Diseased leaves	4	2	100	7.78	13.48	9.39	26.45	17.11	.....	.....
14	do	do	20	½	100	7.78	13.88	10.00	26.79	21.82	.....	.....
15	do	do	20	8	100	7.78	17.41	5.44	.....	.....	1.77	18.99
16	May 6, 1916	do	4	2	100	3.88	11.51	13.04	26.12	14.63	.....	.....
17	do	do	20	½	100	3.88	10.74	Lost.	18.89	.....	.....	.....
18	do	do	20	8	100	3.88	13.23	9.51	.....	.....	5.09	27.54

RESULTS EXPRESSED IN PERCENTAGE OF THE TOTAL NITROGEN OF THE SPINACH MATERIALS

1	Dec. 1, 1915, and Jan. 21, 1916	Healthy entire plant	4	2	52.85	3.22	7.00	3.03	13.71	9.90	.....	.....
2	do	do	20	½	52.85	3.22	8.62	3.41	12.91	10.89	.....	.....
3	do	do	20	8	52.85	3.22	11.72	2.47	.....	.....	2.47	9.17
4	do	Healthy leaves	20	8	50.64	2.55	11.37	2.74	.....	.....	3.79	5.34
5	do	do	20	11	50.64	2.55	12.00	2.54	.....	.....	.....	.....
6	do	do	20	½	50.64	2.55	8.89	2.65	15.25	9.60	.....	.....
7	do	do	4	2	50.64	2.55	7.09	3.80	15.11	9.82	.....	.....
8	do	do	20	½	50.64	2.55	9.20	3.63	13.88	9.69	.....	.....
9	do	do	4	2	50.64	2.55	6.79	3.25	14.21	9.16	.....	.....
10	do	Diseased entire plant	4	2	52.40	4.30	7.06	5.52	14.39	9.87	.....	.....
11	do	do	20	½	52.40	4.30	7.70	3.70	14.56	11.56	.....	.....
12	do	do	20	8	52.40	4.30	9.17	3.02	.....	.....	1.68	8.11
13	do	Diseased leaves	4	2	52.72	4.11	7.10	4.95	13.95	9.02	.....	.....
14	do	do	20	½	52.72	4.11	7.31	5.27	14.12	11.50	.....	.....

15	.....do	.....do	20	8	52.72	4.11	9.17	2.87	.....	.....	0.93	10.02
16	May 6, 1916	.....do	4	2	50.74	2.05	5.33	6.26	12.53	7.42	.....	.....
17	.....do	.....do	20	½	50.74	2.05	4.96	Lost.	9.06	.....	.....	.....
18	.....do	.....do	20	8	50.74	2.05	6.16	4.56	.....	.....	2.59	15.43

RESULTS EXPRESSED IN PERCENTAGE OF OVEN-DRIED SPINACH MATERIALS

1	Dec. 1, 1915, and Jan. 21, 1916	Healthy entire plant	4	2	2.53	0.154	0.336	0.15	0.66	0.47	.....	.....
2	.....do	.....do	20	½	2.53	.154	.416	.16	.62	.52	.....	.....
3	.....do	.....do	20	8	2.53	.154	.566	.12	.....	.....	0.117	0.433
4	.....do	Healthy leaves	20	8	2.57	.129	.581	.14	.....	.....	.195	.265
5	.....do	.....do	20	11	2.57	.129	.611	.13	.....	.....	.....	.....
6	.....do	.....do	20	½	2.57	.129	.451	.13	.77	.49	.....	.....
7	.....do	.....do	4	2	2.57	.129	.361	.19	.77	.50	.....	.....
8	.....do	.....do	20	½	2.57	.129	.471	.18	.70	.49	.....	.....
9	.....do	.....do	4	2	2.57	.129	.341	.16	.72	.46	.....	.....
10	.....do	Diseased entire plant	4	2	1.86	.152	.248	.20	.51	.35	.....	.....
11	.....do	.....do	20	½	1.86	.152	.268	.13	.52	.41	.....	.....
12	.....do	.....do	20	8	1.86	.152	.328	.11	.....	.....	.061	.279
13	.....do	Diseased leaves	4	2	2.22	.174	.296	.21	.59	.38	.....	.....
14	.....do	.....do	20	½	2.22	.174	.306	.22	.60	.49	.....	.....
15	.....do	.....do	20	8	2.22	.174	.386	.12	.....	.....	.052	.398
16	May 16, 1916	.....do	4	2	1.56	.063	.167	.19	.38	.23	.....	.....
17	.....do	.....do	20	½	1.56	.063	.157	Lost.	.28	.....	.....	.....
18	.....do	.....do	20	8	1.56	.063	.187	.14	.....	.....	.078	.482

As will be seen by reference to Table VIII, column 12, the percentage of peptid nitrogen is usually higher in the healthy tissues than in the diseased tissues.

The diseased spinach collected in May, 1916, is strikingly different from the diseased spinach gathered in December, 1915, and January, 1916. An examination of Table VIII shows that the percentage of nitrogen in the form of acid amids and basic nitrogen is smaller in the former than in the latter. This, together with the fact that the protein nitrogen of the May sample is higher than that of the December and January samples (see Table IV) points to the former as being in a riper state whereby the acid amids and basic-nitrogen compounds have preferably been used by the plant for building up protein substance.

The results for residual soluble nitrogen in which the different fluctuations of the various constituents (ammoniacal nitrogen, acid amid nitrogen, etc.) are reflected, range from 10 to 17 per cent in the case of the healthy spinach, and from 15 to 19 per cent in the case of the diseased materials. The May sample has as much as 27 per cent, calculated on the total soluble nitrogen.

SUMMARY

(1) It has been shown (p. 381–384) that carbohydrates accumulate in the leaves of plants affected with the spinach-blight in considerably greater quantity than in normal leaves.

(2) In this paper it is shown that the accumulation is not due to the inability of the diseased plants to make proteins. Although these constituents are found in the tops of the diseased plants in a somewhat smaller percentage calculated on the dry weight of the material than in the normal tops, the proteins make up a larger proportion of the total nitrogen in the diseased than in the healthy material. The proteins in the roots of diseased plants exceed those found in the roots of normal plants, both in reference to the dry weight of the material and to the total nitrogen.

(3) Spinach-blight is physiologically characterized by retarded growth, and a lower moisture content. This seems to be due to the fact that the rapidly growing normal tissues are comparatively rich in water and poor in complex organic compounds, such as proteins, etc.

(4) The lower percentage of total nitrogen and of acid amid nitrogen in the diseased material can best be explained by the assumption that denitrification takes place in those tissues, whereby a part of the nitrogen may be lost either as elementary nitrogen or in the form of ammonia.

(5) The reason for the higher proportion of ammoniacal nitrogen in the diseased material than in the corresponding healthy tissues would be sought in the processes of denitrification, whereby a part of the nitrites is further reduced to ammonia.

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FURTHER STUDIES ON BRISKET DISEASE

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INTRODUCTION

A previous publication[1] of this Station presented a preliminary report of a peculiar dropsical condition found among cattle in the mountains of Colorado which the stockmen call "brisket disease." Some of the more technical studies made previous to the publication of that report were purposely omitted from it, but are given here, with the addition of such observations as we have been able to make since that time.

Altogether we have studied 45 cases, more or less completely, which form the basis of this paper. Reports from New Mexico and Wyoming indicate the existence of disease in that part of this country, but we have never been able to definitely locate it in the high altitudes of any other country. Dr. E. Hess, cattle pathologist, of Berne, Switzerland, informs us that he knows nothing of the disease in that country.

CONDITION OF HEART

The heart, being suspected early as the organ at fault, came in for a considerable share of attention. As stated in a former publication, it is usually very large, flabby, and rather misshapen. Plate 28, B, shows a normal and a diseased heart from two 4-months old calves of approximately the same weight. The normal heart weighed 1½ pounds, while the one from the calf dead of brisket disease weighed 3¼ pounds.

Being anxious to determine whether the hearts of animals raised at high altitudes actually weighed more than those at sea level, a series of hearts were weighed at three packing centers: Denver, Colorado; San Francisco, California; and Fort Worth, Texas. The weighings at Denver were made by Dr. E. W. Alkire, those at San Francisco by Dr. E. A. Meyer, and those at Fort Worth by Dr. O. W. Seher, the two last-named being veterinary inspectors of the Bureau of Animal Industry. Special instructions were given the men so that the trimming might be done in the same manner, and it is believed the results are properly comparable. The hearts were split in such a way that the four cavities were laid open and the vessels were trimmed close to the organ. In most instances a portion of the top of the left auricle was removed. The results of these weighings are given in Table I. It is not considered necessary to give in detail the other characters of the disease, except to say that the animals show generalized edema and enlarged and sclerozed livers such as would be expected in cardiac weakness (Pl. 28, A; 29; 30).

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TABLE I.—*Results of weighings of the hearts and carcasses of cattle at Denver, San Francisco, and Fort Worth*

Lot No.	Killed at—	Raised at—	Altitude.		Number of animals.	Sex.	Weight of carcass.			Weight of heart.			Average weight per 1,000 pounds of carcass.
			Summer.	Winter.			Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	
			Feet.	*Feet.*			*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
1	Denver	Gunnison, Colo.	9,000 to 10,000	8,000	123	Female	713	406	554	6.375	3	4.359	7.985
[a] 2	do	Whitewater	9,000	6,000	4	do	599	464	502	6.500	4.500	5.625	10.807
3	do	Carbondale	8,500 to 9,000	6,700	39	Steers	771	485	603	6.250	3.500	4.413	7.321
4	do	do	Above 8,000		7	Female	726	487	600	5.625	4.125	4.929	8.213
5	do	Pagosa Springs	do		38	do	734	462	551	5.625	3.500	4.270	7.750
6	do	Carbondale, Colo., 16; Rifle, Colo., 1	do		17	do	594	393	518	5.000	3.875	4.353	8.402
[a] 7	do	Falcon, Colo.	6,800	6,800	9	Cows	756	492	551	3.875	3.125	3.561	6.501
8	San Francisco		300	300	55	Steers			669	5.688	3.375	4.590	6.726
9	do		300	300	41	do	753	485	611	5.375	3.500	4.290	7.021
10	Fort Worth	Blessing, Tex.	44	44	42	do	412	308	349	3.500	1.750	2.500	7.163
	Total, or average high altitudes: 1, 3, 4, 5, 6, Denver				224				561			4.371	7.791
	Total, or average low altitudes: 8, 9, 10, San Francisco and Fort Worth				138				554			3.829	6.912
	Difference in favor of high-altitude animals											.542	.879

[a] Not counted in the summary.

Lots 2 and 7 were not included in the summary for the following reasons:

Lot 2 consisted of only four animals, of which No. 1 had a carcass weight of 539 pounds, with a heart weight of 6.5 pounds. The heart was clearly pathological; therefore it was not thought proper to include the lot.

Lot 7 included 9 cows from Falcon, Colo. This lot is of some interest because the hearts averaged lower than either those from San Francisco or Fort Worth, but since the animals were neither from an extremely high nor a very low altitude they were not included in the summary.

It will be seen from Table I that heart weighings were made on 224 cattle raised at high altitudes and 138 raised near sea level. The animals from high altitudes averaged 9 pounds heavier in carcass weight and had hearts averaging 0.542 pound heavier. On the basis of 1,000 pounds of carcass weight, the only proper one for comparison, there was a difference of 0.879 pound in favor of the animals from high altitudes. This number of weighings is probably too few on which to base a conclusion, but the results seem to be in accord with the observations of others made on the subject, and also with what one may reasonably expect, that these animals have heavier hearts than those raised near sea level.

Heger and Meyer, working with guinea pigs and rabbits kept at known air pressures, found the weights of the hearts as shown in Table II.

TABLE II.—*Weights of hearts of guinea pigs and rabbits, according to Heger and Meyer*

Animal.	Air pressure.	Average weight of animals.	Average weight of heart.	Weight of heart per 1,000 gm. of body weight.
	Mm.	*Gm.*	*Gm.*	*Gm.*
21 guinea pigs	765	529	3.88	7.334
	580	448	3.85	8.594
	500	445	3.42	7.686
15 rabbits	765	892	4.92	5.528
	500	870	5.76	6.620
	217	1,224	9.75	7.965

They conclude in the following language:

From the experiment it appears that the thinning of the air had the effect of increasing the weight of the lungs and heart, which was especially true of the rabbit. The increase of weight is, in several cases, considerably more for the heart than the lungs.

EFFECT OF FEED ON BRISKET DISEASE

In order to determine whether the feed or anything in it was the causative factor in the brisket disease, several animals were shipped to the Colorado Experiment Station, where they were fed on hay that had been raised in the high altitude of the South Park district. This hay was of the typical South Park wire-grass, and was obtained from a Den-

ver firm, who informed us that it came from that district. The animals shipped for this determination received no treatment other than ordinary care and got no other food than the hay. Abundant water was given.

Following is a detailed account of these cases:

CASE 33.—Red heifer, 1 year old; raised at Jefferson, Colo., altitude 9,500 feet; arrived at Fort Collins on January 13, 1915. Was very dull, listless; the brisket was somewhat swollen; the abdomen was greatly distended; diarrhea profuse; irregular and rapid heart; respiration rapid and difficult, with grunting. An occasional moist cough was noticed.

She was hauled to the Station stables and given South Park hay and water. She ate not to exceed 5 pounds of hay during the next seven days, her appetite being practically gone (Pl. 28, C). She gradually grew worse and died on January 21. The postmortem examination revealed typical lesions of brisket disease.

CASE 34.—A calf 6 weeks old was shipped on February 13, 1915, by express from Jefferson, Colo., altitude 9,500 feet, arriving at Fort Collins on the 14th. He was in a moribund state on arrival and died on the night of the 14th without eating anything after being delivered at the Station.

CASE 35.—Red-and-white male calf, 6 months old, shipped on March 22, 1915, by express from Woodland Park, Colo., altitude 9,000 feet. The calf had been ill for two weeks previous to shipping. The owner had lost four others with the same trouble. On arrival at the Station he was thin in flesh, and weak but not dull; the brisket was slightly swollen, and the abdomen was enlarged. His appetite was good, and the feces were normal. On South Park hay and water he gradually improved, so that on the 27th the brisket became normal and on the 29th the abdomen had returned to usual size. In all respects the calf was normal, except that he was thin in flesh. He was kept under observation for two or three months, became fat, and finally was sold.

CASE 38.—A 4-months-old heifer calf; shipped from Jefferson, Colo., and arrived at Fort Collins on October 3, 1915. She had a rather severe diarrhea, but there was no swelling of the brisket and not much enlargement of the abdomen. She was placed on South Park hay, but, as she would not eat it, alfalfa was substituted for two days, after which she was given the South Park forage. Diarrhea continued for six days, when the feces became normal, and the calf improved so that she was sold on November 2, 1915, in good condition.

CASE 39.—Hereford heifer, 6 months old, shipped to Denver from Jefferson, Colo., and arrived at the former place on October 11. When seen on that day, she was very dull, the brisket were badly swollen, and she was grunting with each breath. Her appetite, however, was good. She remained in Denver until the 18th, when she was shipped to Fort Collins, arriving there on the 19th. The brisket was still swollen, although much reduced. She was placed on the South Park hay and continued to improve, so that on October 23 the swelling had entirely disappeared. She became normal and was sold on November 2.

CASE 40.—Yearling Shorthorn steer; shipped to Denver with the preceding case. Quite thin; had diarrhea, but no swelling of brisket. Arrived in Fort Collins on the 19th, was placed on South Park hay, improved rapidly, and was sold in normal condition on November 2.

CASE 41.—A 2-year-old Hereford steer; shipped with cases 39 and 40 and treated in the same manner. This steer was very thin, had a diarrhea, and was scouring badly. He gradually improved on the South Park hay, but did not put on much flesh until spring. He gained in strength, and the scouring stopped at about the tenth day after arrival at the Station. In March he was sent to pasture, and there died of tympanites on March 29.

These six cases were fed the high-altitude South Park hay in order to determine whether the feed was a factor. The first two animals died without eating enough of the hay to determine its effect, but the other four improved and finally recovered on it. Therefore, we are led to believe that the change of altitude and not the change in feed is the essential factor in the recovery of animals from this disease on being shipped to the lower levels.

SUMMARY

Our observations tend to show that normal animals living in a high altitude have a heavier heart than those living near sea level; that animals affected with brisket disease had dilated, flabby, and heavy hearts; that they have a high percentage of red corpuscles; that they show generalized edema and enlarged and sclerosed livers, such as one would expect in cardiac weakness; that they usually recover when shipped to lower altitudes, but seldom do if they remain at the higher levels; and that the feed is not a factor; that animals from low altitudes are more often affected than natives; that calves sired by bulls from low altitudes are more likely to be affected than those sired by native bulls; that the higher the altitude the more prevalent is the disease.

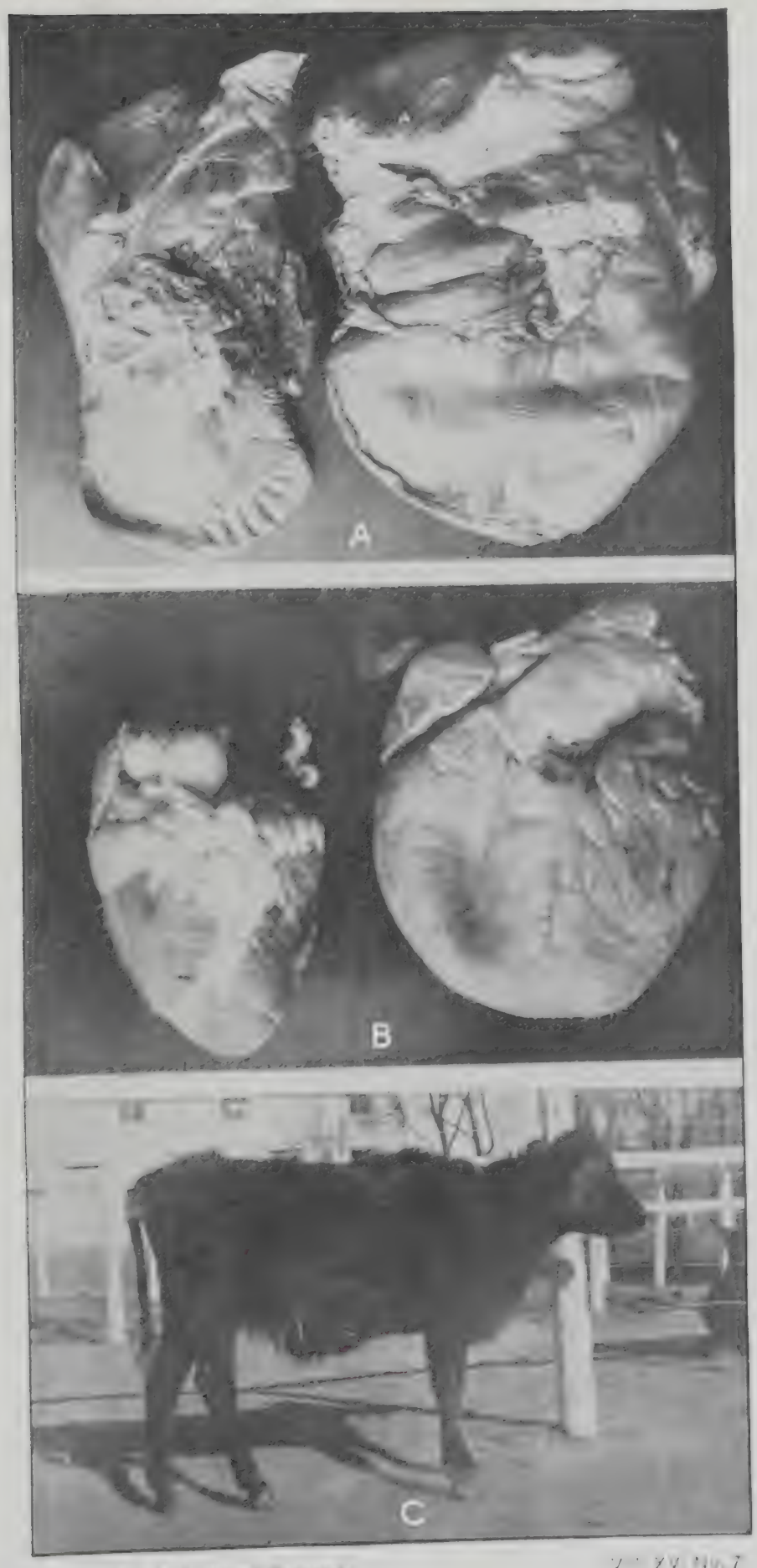
We therefore have no hesitancy in concluding that the malady is due to failure of acclimatization at high altitudes. The remedy lies not in drugs, but in breeding a hardier strain of cattle which can accustom themselves to the rigorous conditions incident to an existence at these extreme altitudes.

PLATE 28

A.—Livers of normal calf and one affected with brisket disease. Same age. Normal liver weighed 4¼ pounds, the diseased 10 pounds.

B.—Hearts of normal animal and one that died of brisket disease. Same age. The normal weighed 1½ pounds, the diseased 3¼ pounds.

C.—Case 33, a heifer showing the characteristic symptoms of the brisket disease.


A
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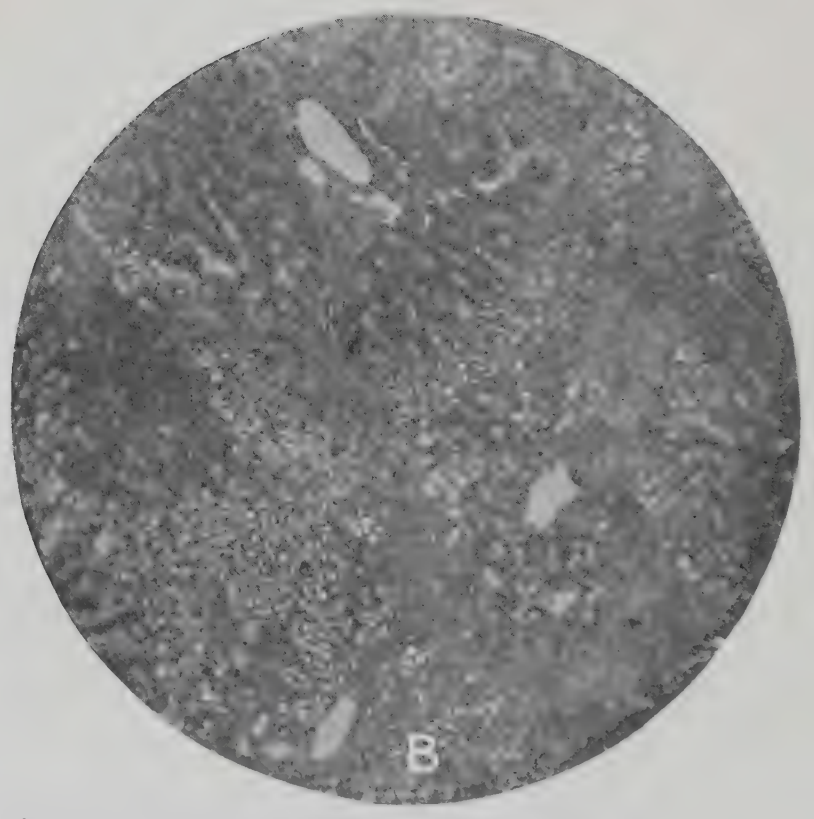

B

PLATE 29

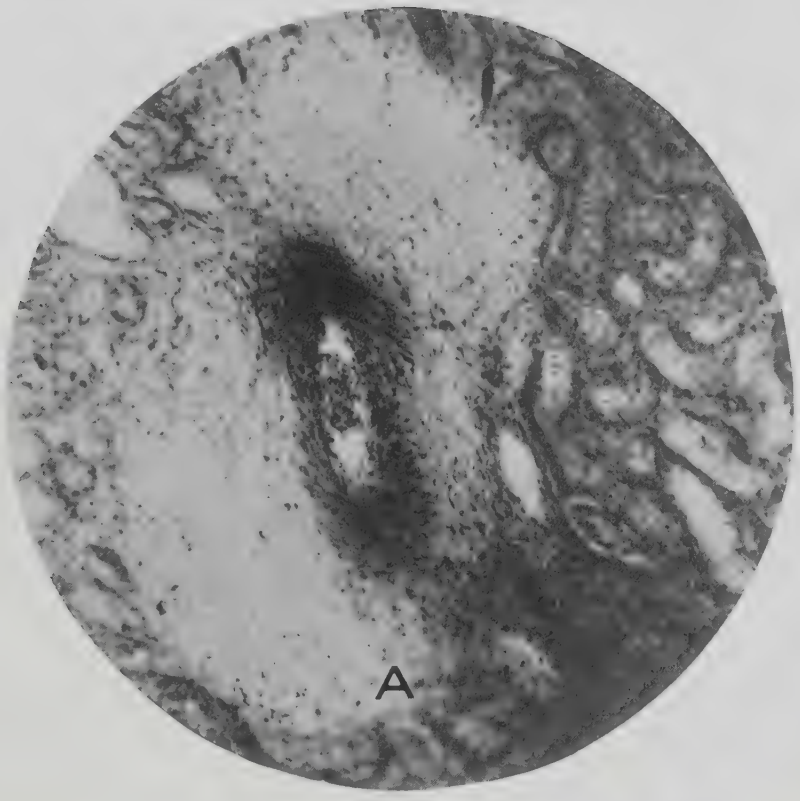
A.—Interlobular connective tissue in the liver of an animal dead of brisket disease. The excessive weight and toughness of these livers seem to be referable to a new formation of fibrous tissue.

B.—Fatty accumulation in the liver in early stage of brisket disease.

PLATE 30

A.—Edema around one of the arterioles in the kidney.

B.—Malpighian body in the kidney of an animal dead of brisket disease. Note that Bowman's capsule is dilated and filled with detritus.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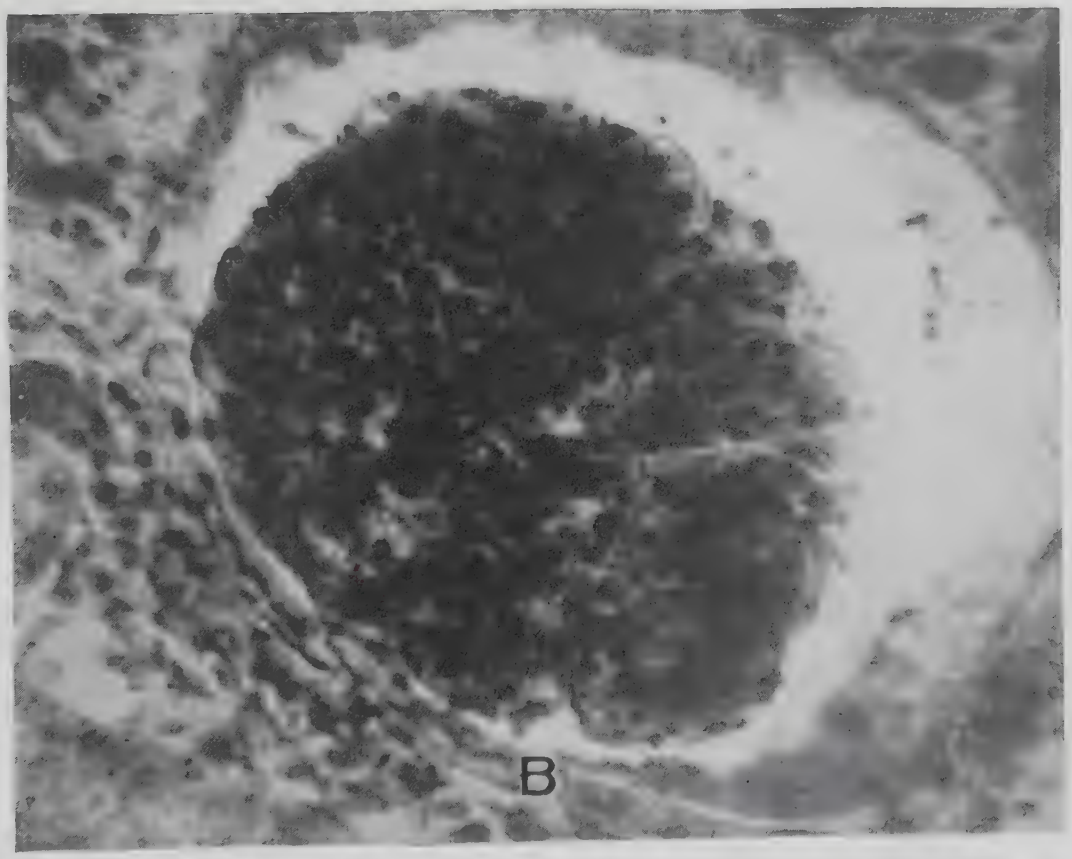
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OBSERVATIONS ON AN OUTBREAK OF FAVUS[1]

By B. A. BEACH and J. G. HALPIN

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INTRODUCTION

Favus is a disease of the skin of animals, man, and poultry. In fowls it begins as a white, scaly deposit on the unfeathered portions of the head and may spread to the feathered parts of the skin, but never extends to the internal organs.

Schönlein in 1841 was the first accurately to describe the disease. Other investigators have since recorded their observations.

Favus is widespread, especially in chickens, which seem to be the most susceptible of all poultry. In Wisconsin, to our knowledge, several severe outbreaks have occurred in the last few years.

CAUSE

The cause of favus is a fungus which has been named *Achorion Schönleinii* after its discoverer. Some variations have been noted in the appearance of the fungus that has been isolated by us. These were due to the stain used and also to the condition of the culture when examined. Young and actively growing cultures treated with methylene blue stain deeply and fairly uniformly. In old, partially dry preparations stained with methylene blue a limiting capsule may be seen. Internal to this capsule is a central protoplasm which is more or less granular. The spores are oblong in shape and are about 10 to 12 μ long by 8 to 10 μ wide.

The processes of growth and reproduction in single spores and groups of two or three were determined by the use of the Barber method.

There is seen during the first few hours a distension of the capsule followed by elongation of the organism. This is probably what Ricketts[2] refers to when he speaks of the club-shaped appearance. After 24 hours, branching mycelia may be seen. They continue to grow for about 48 hours, when, if conditions are right, the hyphæ break up into spores beginning at the far end and advancing toward the parent mycelium. These observations were made by flooding isolated spores with a drop of naturally sterile horse serum. The preparations were incubated at 37° C. except during observation. In all cases it took about 48 hours for the cycle of growth to be completed—that is, for one spore to produce daughter spores. The organism in its morphology and method of reproduction bears considerable resemblance to some of the oidia.

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[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] RICKETTS, Howard T. OIDIOMYCOSIS (BLASTOMYCOSIS) OF THE SKIN AND ITS FUNGI. *In* Jour. Med. Research, v. 6 (n. s. v. 1), no. 3, p. 373–547, pl. 22–33. 1901. Bibliography, pp. 538–544.

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Particles of the honeycomb-like crust were broken into small fragments and shaken vigorously in sterile water to disintegrate the material. Plates of potato-serum agar, acidified by the addition of 5 drops of an *N*/10 hydrochloric-acid solution to 10 cc. of the medium, were then inoculated with the above material. Forty-eight hours later there appeared on the surface of the media grayish-white, cottony-like colonies, which, when transferred to serum agar slopes at room temperature, gave a granular whitish growth along the stroke of the needle. After 10 to 14 days the whole surface presented a frosted white appearance and became uneven. The growth appeared to heap up in places, forming a finely granular wrinkled appearance. It also grew down into the medium. With age the growth assumed a yellowish tinge.

The following results were noted after seeding other media: Gelatin was very slowly liquefied; growth on potato was very slow; litmus milk became slightly reddish in tint, but was not coagulated; growth in bouillon was slow, but greatly accelerated if 0.5 per cent of raw horse serum was added. In this medium the growth first occurred as a membranous mass on the surface, followed by a sedimentation and slight turbidity. The particles were of a flocculent nature. The bouillon did not assume the uniform turbidity seen in bacterial growth.

ANATOMICAL CHANGES

Microscopic examination of pieces of affected comb that had been hardened in alcohol, embedded in celloidin, and sectioned revealed the organism in the epithelial layers and also in the cutis. Dead tissue cells, leucocytes, and bacterial cells were present in considerable numbers. The fungi appeared to be assembled in groups of about 10, but in one case as many as 50 were observed in a group. Branching forms were numerous. Growth in the tissue apparently took place by branching. It is possible, however, that spores were formed, although none were seen in any of the specimens studied. No abscesses nor ulcers were seen in any of the cases.

Favus manifests itself clinically in the form of a dry, white, scaly deposit, which usually appears first on the comb and then spreads to the face and wattles. In advanced cases the feathered portions of the body are attacked to such an extent that the skin is denuded of feathers (Pl. 31). As long as the disease remains localized about the head the general health is unimpaired. In such cases the egg production does not seem to be interfered with. Where both the neck and body are involved, constitutional changes may be noted. These disturbances are probably the result of absorption from the necrosed epithelium and of bacterial invasion. There is evidently no toxin produced by the fungus.

ENZOOTIOLOGY

The first case of favus that came to our attention was in the flock on a farm not far from the poultry yards of the experiment station. These chickens were allowed to run at large, but could not come in contact

with birds in the experiment station yards, as these were fenced. The case mentioned above was discovered in October, 1913, and the bird taken to the university poultry building. Later it was placed with some cockerels and pullets that were in quarantine and kept with them until April. In April all the birds were sold. Very careful observations were made, but none of the birds showed any signs of favus except the cock bird previously mentioned.

On July 14, 1914, breeding cocks from the Station flocks were placed in the house and yards formerly occupied by the favus case. On October 12, 1914, two of these cocks showed distinct lesions of favus. These birds were isolated and treated with iodin and strong soap solution. After many treatments they were apparently cured. Subsequently the disease occurred in pens where these two males were placed and also in other houses on the research plant, where these birds had never been.

Two pullets were taken from one of the infected pens on December 26, 1914, and put with a clean flock about half a mile away. Careful observations were made, and on February 20, 1915, one of these pullets showed distinct lesions of favus.

A careful watch was kept on the flock where the first case was found. In fact, one of the writers has handled all the birds on this farm and examined each bird carefully three times since the first case was discovered, but has never been able to detect any other cases there. The owner, who is quite a careful observer, states that he had never observed a case prior to the cock bird which, we believe, introduced the original infection. Furthermore, this bird was raised on the farm. No new stock had been introduced on that farm for at least three years, and, so far as known, no other stock mingled with this flock.

TREATMENT

After much experimentation with lysol, tincture of iodin, and other recommended remedies it was found that an ointment made of formaldehyde and vaseline was far more efficient than any of the other preparations. This ointment may be prepared by placing vaseline in a Mason fruit jar and heating it in water until the vaseline melts. Then 5 per cent by weight of commercial formaldehyde is added. The cover of the jar should be tightened immediately and the mixture shaken until the vaseline hardens. One or two applications of this preparation rubbed thoroughly into the lesions usually will suffice. (See Table I.)

TABLE I.—*Results of the treatment of favus with iodin and the vaseline-formaldehyde ointment*

Preparation used.	Number of cases treated once.	Number of cases treated twice.	Number of cases treated three times.	Number of cases treated four times.	Number of cases treated five times.
Tincture of iodin	62	60	60	54	45
Ointment	50	2	0	0	0

By referring to Table I it will be seen that it was necessary to treat 45 cases five times with tincture of iodin in order to get satisfactory results. Thirty of these birds were subsequently treated once with the vaseline-formaldehyde ointment, but are not included in the table. The two cases that required a second treatment with the ointment were very severe and of long standing. All of the cases in this group made a rapid recovery.

EXPERIMENTAL STUDIES

Experiments to determine the method of infection in favus were carried out as shown below. It should be stated that attempts to infect a fresh, bleeding wound were unsuccessful.

HEN 138. Scarified small area on comb and wattles. The following day a small quantity of material from an infected bird was instilled beneath the scab. Five weeks later the disease was well under way. Recovery almost complete in six months.

HEN 610. Infected same as above. Infection very apparent three weeks later. This case grew steadily worse, but finally responded to treatment.

HEN E146. Infected same as above. Infection apparent 15 days later. Hen destroyed and comb used for culture and sectioning.

HEN 627. Fed large quantities of favus material. No lesions appeared.

HEN 669. Fed same as above with negative results.

HEN 10. Fed same as above with negative results.

HEN E106. Area under left wing scarified, scab removed and favus material instilled the following day. No lesions appeared.

HEN 24. Same as above with negative results.

HEN E97. Favus material in salt solution was injected into the vein on under side of wing. No lesions appeared.

HEN 25. Area on comb scarified, the following day scab was removed and small quantity of culture isolated from E146 instilled. Eleven days later infection very apparent. Hen finally destroyed.

HEN 64. Handled same as above. Six weeks later growth apparent. This hen recovered about 12 weeks after it had been infected.

CONCLUSIONS

These experiments, so far as they go, show that—

(1) Favus is primarily a wound-infection disease of the unfeathered parts of the head.

(2) It occurs usually as an enzootic.

(3) An ointment composed of vaseline and formaldehyde is an effective remedy.

(4) Infection by the digestive tract is impossible.

(5) Intravenous inoculations are incapable of starting infections.

(6) The organism isolated and studied by us is specific, as shown by the fact that typical cases of the disease were produced in hens inoculated with laboratory cultures.

PLATE 31

Bird affected with favus: A pronounced case showing involvement of the com
face, and neck.

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A CONTRIBUTION TO THE BIOLOGY OF FRUIT-FLY PARASITES IN HAWAII

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INTRODUCTION

With the termination of an intensive study of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) in Hawaii in February, 1916, by the Bureau of Entomology, a general investigation of the biology, interrelation [1] and economic value of introduced parasites of this fruit fly was begun. The results of this investigation are herewith presented.

The natural enemies of the Mediterranean fruit fly now contributing toward its control in Hawaii are three species of Opiinae—viz, *Diachasma tryoni* Cameron, *Opius humilis* Silvestri, and *D. fullawayi* Silvestri, one species of Eulophinae, *Tetrastichus giffardianus* Silvestri, and an ant, *Pheidole megacephala* Fabricius. Two other parasites occasionally reared from the fruit fly are *Opius fletcheri* Silvestri, normally a melon-fly parasite, and *Pachycrepoideus dubius*, a dung-fly parasite. At present parasitism by these two parasites is not important, and may be more accidental than normal. The opiines and the eulophid are strictly larval parasites. The ant is important as a predacious enemy of the larvæ and to a lesser extent of the pupæ. The pteromalid *Pachycrepoideus dubius* attacks the pupa. There is striking similarity in habit, structure, and development among the opiines under discussion, and in view of this the species now most abundant in Hawaii, *Diachasma tryoni*, will be dealt with the most completely, to be followed by notes on the special features of the two other important opiine species together with comparisons with the species *tryoni* in sufficient detail to give a thorough conception of the biology of each.[2]

[1] For previous studies on fruit-fly interrelations in Hawaii, see Pemberton and Willard (*6*). [Reference is made by number (italic) to "Literature cited," p. 465.]

[2] For the original descriptions and history of the introductions of the important species above mentioned, see BACK, E. A., and PEMBERTON, C. E. (*3*).

For detailed records on the extent of parasitism in Hawaii by these species see Back and Pemberton (*1, 2*) and Pemberton and Willard (*5*).

DIACHASMA TRYONI

Diachasma tryoni Cameron was first observed in New South Wales in 1908 and was described in 1911. It was introduced into Hawaii from New South Wales by Silvestri in May, 1913. It soon became definitely established and by 1916 its importance as a parasite and its ready adaptation to Hawaiian conditions were demonstrated clearly. During 1917 it excelled the work of the other introduced parasites

DESCRIPTION AND LIFE HISTORY

EGG

The egg (fig. 1, 2) is cylindrical, translucent white, with smooth, glistening surface, drawn out at each end into a short, rounded protuberance and when first deposited is surrounded by a thin, transparent membrane, possibly the exochorion. This membrane adheres to the conformity of the egg but does not tightly inclose it. The egg is faintly concave ventrally and distinctly convex dorsally. The protuberance on the cephalic end is slightly broader and shorter than is that on the caudal end. At deposition the egg averages 0.45 mm. in length, including the enveloping membrane, its greatest width being about one-sixth of the length. When fully developed the egg averages 0.65 mm. in length and is about one-fourth as broad as long. During development the outer enveloping membrane is ruptured and entirely separated from the egg proper. At maturity each end of the egg stands out as an abrupt, broad tubercle. The embryo is then clearly revealed by transmitted light.

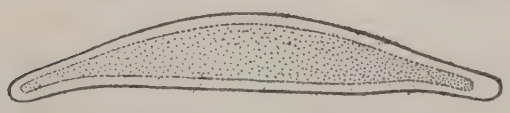

Fig. 1.—*Diachasma tryoni:* Egg just laid; length 0.48 mm.

Although the eggs are deposited only slightly beneath the surface of the larval skin, they are invisible even under strong sunlight and magnification. The duration of the egg period can be determined only by dissections of host tissue at frequent intervals to locate the eggs and by the use of numbers of well-parasitized larvæ known to have been oviposited in for a short and definite period. The egg stage in Honolulu (Table I) was found to last from 54 to 73 hours, the variations depending upon fluctuations in temperature. As the average incubation period is about 2½ days, the exact number of hours required for the development of eggs deposited in the morning is less than is that where eggs are deposited in the late afternoon. Eggs deposited in the morning hours occupy parts of three days and two nights before hatching, and thus develop under a somewhat higher average temperature than would obtain in the case of eggs deposited in the afternoon or early evening.

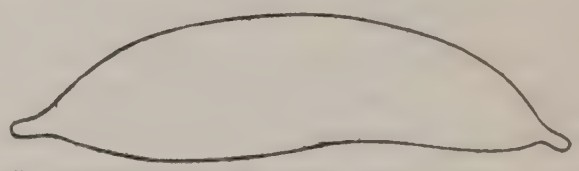

Fig. 2.—*Diachasma tryoni:* Egg mature; length 0.65 mm.

These pass through parts of three nights and two days as opposed to three days and two nights and would thus be subjected to somewhat lower average temperatures. This will explain some of the misleading variations shown in Table I.

TABLE I.—*Duration of the egg stage of Diachasma tryoni in Honolulu*

Number of eggs under observation.	Eggs deposited.	Eggs hatched.	Average duration of egg stage.	Mean temperature.
			Hours.	°*F.*
24.....	Jan. 4, 11 a. m. to 1 p. m...	Jan. 7, 11 a. m. to 2 p. m...	73	71.0
138.....	Mar. 21, 11 a. m. to 1 p. m	Mar. 24, 9 a. m. to 10 a. m.	70	73.2
130.....	Apr. 16, 9 a. m. to 10 a. m.	Apr. 18, 10 p. m. to 11.30 p. m.	62	74.2
73.....	May 31, 3 p. m. to 3. 30 p.m.	June 3, 6 a. m. to 8 a. m...	64	74.0
189.....	July 6, 11 a. m. to 2 p. m..	July 8, 6 p. m. to 10 p. m..	56	75.9
38.....	July 7, 2 p. m.....	July 9, 9 p. m. to 11 p. m..	56	76.0
47.....	Oct. 5, 8 a. m. to 10 a. m..	Oct. 7, 1 p. m. to 4 p. m...	54	76.6

The fully developed egg is so swollen and the membrane so thin that actual emergence of the larva from the egg is sudden, and rather an explosive process. Many eggs on hatching have been under observation. The egg membrane is suddenly ruptured longitudinally, probably by the movements of the larva within, and the larva floats out without apparent effort into the semiliquid medium of the host surrounding it. The egg may hatch while the host is still in the active, feeding, larval stage or it may hatch after the puparium is formed and the complete histolysis of larval tissue has taken place. No important histogenetic action occurs in a puparium containing a parasite egg or larva. The parasitized host larva feeds and develops to maturity even though heavily superparasitized, leaves the fruit normally, and forms a perfect puparium in the usual manner. The complete histolysis of the larval tissues within the puparium then follows, but here all fly development ceases. Henceforth the content of the puparium is but a liquid mass containing the broken-down bits of larval tissue and the rapidly developing parasite larva. The death of the host then may be said properly to occur at the cessation of histolysis in the newly formed puparium. From over 3,000 parasitized puparia opened during 1916 and 1917 no single case was ever noted in which a perfect or even partially formed fly pupa occurred.

LARVA

The larva undergoes many interesting phases in the processes of transformation. When first hatched, it is about 0.85 mm. long. This is the most active period in the larval life. At this time it is so markedly different from the later instars that it appears to simulate the larval structure and habits of an ancestral type. It usually hatches while the host is still in the larval stage. The parasitic larva then lies in a well-

organized body, wherein its food, which seems largely the fat body of the host, is in a semisolid state, in part isolated into definitely compacted masses. The larva is lodged in an area which is well organized in muscular, digestive, nervous, and respiratory structures, all of which combine to interfere with its freedom of action. Special characters, not appearing in the succeeding instars, are peculiarly adapted to this stage. The head is large, heavily chitinized and brownish, and bears a pair of sickle-like mandibles, with bases broadly separated and capable of wide movement and quick action. Above the mandibles and seemingly on the labrum is a pair of small, short antennal structures, which are frequently extended and withdrawn in a rapid, vibratory manner as the larva feeds and moves about. On the cephalic edge of the chitinized ventral portion of the head is borne a pair of pointed teeth, well separated and together forming a distinct letter U with the basal connecting line more or less straight. This lies below and directly between the bases of the mandibles (fig. 3). Its shape affords the best character for differentiating the larva of this species from the newly hatched larva of *Diachasma fullawayi* or that of *Opius humilis*. A clearly defined, simple tracheal system is present (fig. 4) and becomes filled with air shortly after the larva has hatched. No spiracles occur, but eight minute, oval swellings can be seen along each main tracheal trunk in body segments 1 to 8. The larva lies strictly within the host, and the air which quickly fills the tracheæ must be obtained by osmosis from the aerated liquid media surrounding it. The tracheæ are filled with air before food has been taken, which shows that the air is not extracted internally from the ingested food. The digestive canal (fig. 5) is a simple, straight tube

FIG. 3.—*Diachasma tryoni:* Cast skin of first-instar larva, showing head characters of first instar and serosal cellular mass still clinging to ventral surface. Length 1 mm.

with short, narrow esophagus, large midintestine occupying the greater bulk of the body and closed caudally, and the short proctodæum terminating with an apparently open, oval anus situated on the ventral surface of the third to the last body segment. The only food taken that is readily visible is the fat of the host. With the development of this instar the midintestine becomes gradually filled and swollen with globules of fat. Newly hatched larvæ generally are found moving about in the fat body and have been dissected frequently from fly larvæ with the mandibles closed into portions of the fat. Though the large, pointed mandibles enable the larva to lacerate tissues other than the fat body, through some unknown influence the delicate vital organs of the host larva seem never to be injured, even in cases of superparasitism when six or eight newly hatched larvæ may be cutting about with their mandibles, either in the separation of food or in the destruction of one another. The first-instar larva moves about by contorting the body, and its movements are aided by gripping fresh tissues coming into contact with the mandibles coincident with the body movements. The brownish, chitinized head can be seen moving within the host larva when under strong light and fair enlargement. The larva is legless, but bears a pair of soft, short, saclike appendages on the ventral side of the body just back of the head (fig. 3, 4). They are incapable of movement and may be gill-like in their function. No tracheæ can be seen leading into them even when examined under high magnification. Extending along the ventral surface of the body, from the back of the head to the tip of the abdomen, is a gelatinous mass of large cells. These are the serosal cells of the egg and adhere to the larva until it molts for the first time (fig. 3). Just before molting the larva becomes greatly engorged with food and has increased to about 1.2 mm. in length.

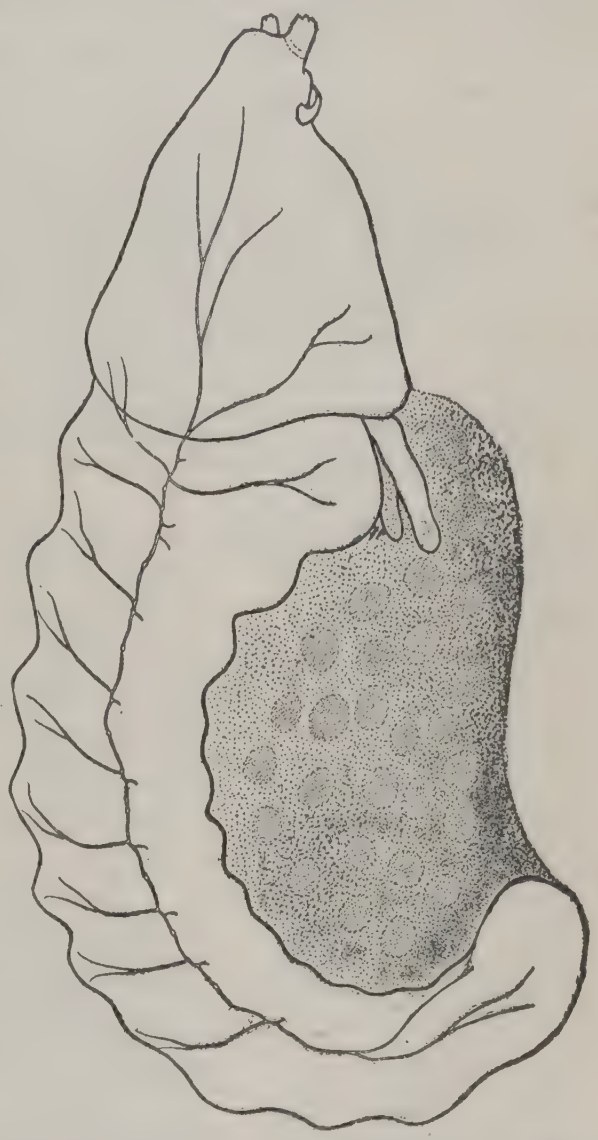

FIG. 4.—*Diachasma tryoni:* Larva of first instar, lateral aspect, showing right main tracheal trunk with branches, and characteristic position and volume of egg serosal cells clinging to ventral surface. Length 1 mm.

The duration of the first instar larva is dependent upon a curious circumstance. The larva never molts until the fruit-fly larva attempts to pupate. Thus, a small fly larva may be stung by the parasite while the larva is in a fruit of dry texture or hard pulp. Usually the larva will not mature or try to pupate until from 6 to 10 days later when in such a fruit. In this case the parasite egg hatches in the usual time (54 to 73 hours) and the parasitic larva remains in the first instar during the long 6- or 7-day period remaining until the host prepares to pupate.

FIG. 5.—*Diachasma tryoni:* Larva of first instar about to molt, lateral aspect showing food canal filled with fat globules and illustrating the beginning of the formation of the meconium. Length 1.5 mm.

Under ordinary circumstances the parasite stings a mature larva, which usually forms a puparium a few days later. Here the parasitic larva hatches about the time the host larva is fully developed and ready to attempt pupation. The first instar in such cases lasts from 36 to 48 hours. The following specific cases were observed during 1917: Two small fruit-fly larvæ, each stung by a female of *Diachasma tryoni* on June 14, still contained first-instar *tryoni* larvæ on June 21. Thirty-one small fruit-fly larvæ, stung by *tryoni* females on August 1, still contained first-instar *tryoni* larvæ when opened on August 8. On August 12 four had formed into puparia by 9 a. m.[1] They were opened on the same day at 4 p. m. and each was found to contain a freshly transformed second-instar *tryoni* larva. The other fly larvæ did not show signs of pupating after several days. Four of them were opened on August 16 and each contained a first-instar *tryoni* larva. The remaining four, still active, were opened August 18 and found to contain a well-developed *tryoni* larva in the first instar. In this series of examinations of the 12 parasitized fly larvæ the first 4 produced *tryoni* larvæ whose first instar lasted about 36 hours, the second 4 contained *tryoni* larvæ whose first instar had already lasted about 6 days and the last 4 contained *tryoni* larvæ whose first stage had already extended about 8 days. The extended time in the duration of the instar was in each case entirely controlled by the delay of the host in attempting to pupate.

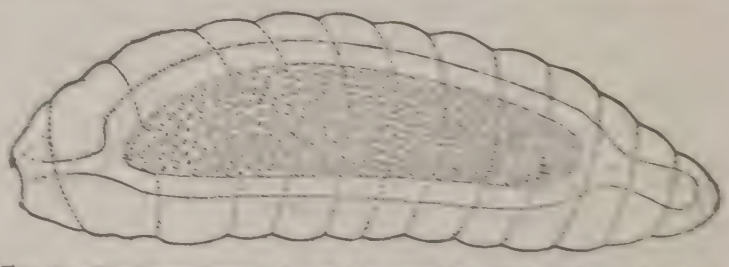

FIG. 6.—*Diachasma tryoni:* Larva in second instar, dorsal aspect, showing general shape of body and food canal. Length 2.4 mm.

[1] All references to clock time refer to "standard time."

When the larva has molted to the second instar, the molted skin can be dissected easily from the fly puparium (fig. 3). In the second instar the larva is greatly changed (fig. 6). The head does not stand out strongly differentiated from the other body segments as in the preceding instar. It is soft, unchitinized, and without pronounced visible characters. The articulations of the 14 body segments can be clearly seen. The body is glabrous throughout. The mandibles (fig. 7) are soft and translucent and can be seen only with difficulty. The weight of a coverglass may easily crush them beyond recognition. They are sharply pointed, short, and about as long as broad, averaging 0.021 mm. in length. Mandibles are not needed in this instar, as the food is composed entirely of fluids, minute globules of fat, and possibly fragments of disintegrated tissue. As the development of the larva progresses the mandibles of the third instar may be seen distinctly pushing into ultimate position at the bases of the mandibles. The larva averages about 1.5 mm. in length in this stage.

FIG. 7.—*Diachasma tryoni:* Mandible of second-instar larva, showing mandible of third instar pushing from within. Length 0.021 mm.

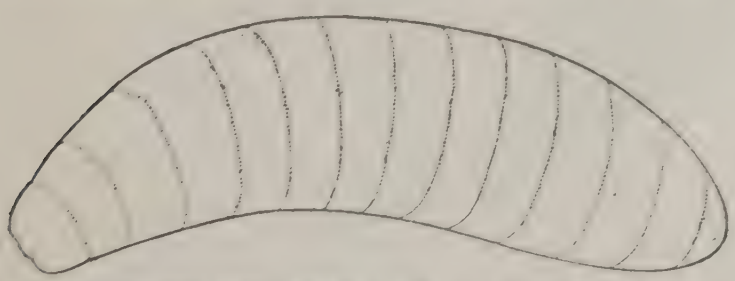

FIG. 8.—*Diachasma tryoni:* Larva of the third instar, dorsal aspect. Length 2.9 mm.

One striking feature in the second instar is the total absence of tracheæ, as careful examinations of more than 100 second-instar larvæ under the highest magnification and best light failed to reveal any evidence of tracheal trunks or branches. In view of the presence of a well-marked respiratory system in the preceding instar, the absence, at this stage, of tracheæ is of distinct interest. As the larva is now immersed in a thin liquid there would seem to be no need for tracheæ. The digestive tube is filled with food, and, as in the first instar, takes the form of the simple midintestine. The oily fat globules of the host which are ingested are conspicuous in this portion of the intestine. This intestine is closed caudally, although the short hind intestine may be seen leading up to it. In this instar the larva is very sluggish, and there is no need for action, considering the accessibility and character of the food. The duration of this stage has not been determined accurately. There is no wide variation in its length, however, such as occurs in the first and fourth instars. The average duration of the second larval instar is about 48 hours. There

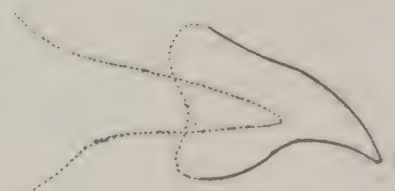

FIG. 9.—*Diachasma tryoni:* Mandible of third-instar larva, showing mandible of fourth instar pushing from within. Length 0.035 mm.

is little to distinguish the third (fig. 8) from the second larval instar, and it is even less pronounced in character The mandibles (fig. 9) are slightly heavier and are about 0.035 mm. in length. Otherwise they are almost identical with those of the previous instar. The mandibles of the forming fourth instar can be seen pushing from within into the bases of the mandibles. There are no hard, darkened or chitinized parts in any portion of the body. As in the preceding instar, no traces of tracheæ occur, but late in the development of this stage the strong, heavy tracheal trunks, branches, and stigmata of the succeeding instar may be seen organizing beneath the skin. The stigmata do not, at any period in the development of the third instar, become opened to the surface, as they are not a developed accessory of this stage, but belong strictly to the succeeding instar. The body is glabrous throughout. Late in the development of this stage the spiny cuticula of the succeeding instar may be seen beneath the integument. No change has been noted in the digestive tract, other than that of a gradual increase in the volume of food ingested and the increased volume of waste matter accumulating in the closed midintestine (fig. 10). This stage averages about 2.4 mm. in length. It still lies immersed in the body fluids of its host, although shortly before molting to the last instar a large part of the body is usually exposed in the hollow puparium. The average duration of the third larval instar will approximate 48 hours.

FIG. 10.—*Diachasma tryoni:* Third-instar larva, lateral aspect, showing digestive canal. Length 3.1 mm.

When the fourth instar is reached, the conditions surrounding the larva have undergone a great change. Much of the liquid and semiliquid contents of the host have been consumed, and within a short time little remains in the puparium but the parasitic larva. To meet this condition, the parasitic larva is possessed from the first of a well-defined tracheal system. Nine large, open stigmata are borne on each side of the second to the eleventh body segments (fig. 11). No stigmata occur on the third segment, however, although a branch from the tracheal trunk on each side leads to points on the surface corresponding in position with the stigmata on the other body segments. A main tracheal trunk extends along each side of the body with a special branch to each stigma and to the dorsal and ventral portion of each body segment. A connecting branch joins the two main trunks posteriorly and anteriorly. With the exception of the head the entire body is closely covered with

short, sharp, curved spines with broad bases (fig. 12, 13). These spines are absent along the line of articulation between each segment. The body averages 3.1 mm. in length and about 1 mm. in width. The characters of the head are strongly developed (fig. 14). A well-defined labrum, heavily pointed mandibles about 0.12 mm. long, with broad rounded bases and brownish chitinization at the tips and bases (fig. 15), distinct maxillæ with short major and minor palpi, and a chitinized labium with palpi, can be distinguished readily. The palpi are only short tubercles. Well-developed, yellowish, tentorial ridges in the head support the mouth parts. The mandibles and tentorial structures of the head are colorless at first. Some hours after the molt they assume the yellowish-brown color that so readily distinguishes this instar from the preceding. The head is about 0.5 mm. in width. As the remaining food consumed by this instar is liquid, the purpose of the well-developed mandibles, which may be vigorously moved, has not as yet been established. No change takes place in the alimentary canal upon the molt to this instar. The larva is very sluggish, though it may bend its body slowly from side to side. It usually lies with its head in the head end of the puparium. Of 76 parasitized fruit-fly puparia opened to determine this point, 64 contained mature *Diachasma tryoni* larvæ in this position. In the remaining 12 the position was reversed. When in the first instar, the larva moves about with its head in no constant direction.

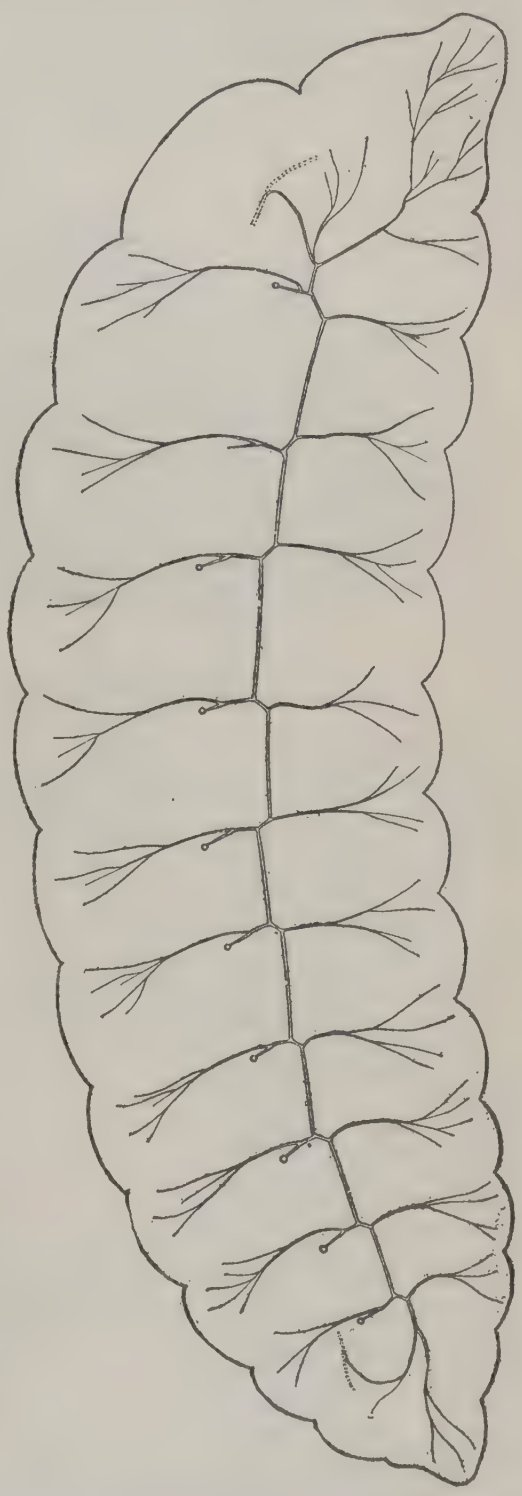

Fig. 11.—*Diachasma tryoni:* Larva of fourth instar, lateral aspect, showing complete right tracheal trunk with branches and stigmata. Length 3.1 mm.

The duration of the mature larval stage is of unusual interest. In Hawaii it may extend from about 6 days to over a year. Larvæ ordi-

narily pupate within 8 or 9 days after the host puparium is formed and the adult parasites emerge in from 5 to 8 days later. A certain proportion of the larvæ reaching maturity each month of the year, however, pass into a dormant state and may remain in this condition for from a few weeks to several months, or occasionally a year. No doubt periods of long drouth and scarcity of host material in Australia, in the localities

Fig. 12.—*Diachasma tryoni:* Mature larva, lateral aspect. Length 3.1 mm.

where this species is probably native, are frequent, and these conditions may have necessitated such an adaptation. This parasite may thus persist under very unfavorable conditions. With its introduction into Hawaii this strong, inherent trait endures, even though climatic and host conditions are ideal for continuous reproduction throughout the year. The hibernating larvæ look much the same as do other mature larvæ, although the body is somewhat contracted, shortened, and slightly paler in color. During the entire period of dormancy the larva is capable of slow movement. The dormant larvæ are not necessarily undernourished individuals. In fact, hibernation has been noted among individuals possessed of large, fully nourished bodies. No great variation in the degree of hibernation has been noted to occur in fly larvæ from any special variety of fruit. The number of males produced from hibernating material greatly exceeds the number of females. Between August, 1916, and July, 1917, 663 males to 118 females emerged from hibernating lots, ranging from 1 to 12 months old.

Fig. 13.—*Diachasma tryoni:* Greatly enlarged view of spines covering surface of body of mature larva. Length, 0.01 mm.

Parasitized fruit-fly puparia placed in dry glass vials or jars yield a much lower percentage in hibernation than do puparia left in the soil

under natural conditions. Thus, in March, 1917, a total of 2,725 French cherries, *Eugenia uniflora*, collected in Honolulu, yielded 1,213 puparia parasitized by either *Diachasma tryoni* or *D. fullawayi*. One-half of the puparia were placed in glass vials and the remainder in a screened box and covered with ½ inch of sand. Of the lot placed in vials 242, or 39.9 per cent, of the larvæ of *Diachasma* spp. hibernated, and of those in the sand 543, or 89.4 per cent, entered hibernation. Again, in September, 1917, during a warm part of the year, a quantity of kamani nuts (*Terminalia catappa*) was collected in Honolulu and placed in a large screened box containing sand, and left in the open air. Of 785 parasitized fruit-fly puparia forming in the sand in this box 271 produced living adults of *D. tryoni* in the usual time, while the remaining 514, or 65.5 per cent, upon examination late in October were found to contain living larvæ of *D. tryoni*. A repetition of this experiment was started in November, 1917. Of 934 parasitized fruit-fly puparia forming in the sand in the box from November 2 to 20, only 69 produced adults of *D. tryoni* in the usual time. The remaining 865 puparia were opened in January, 1918, and all were found to contain living larvæ of this species. This is an average hibernation of 92.6 per cent and repre-

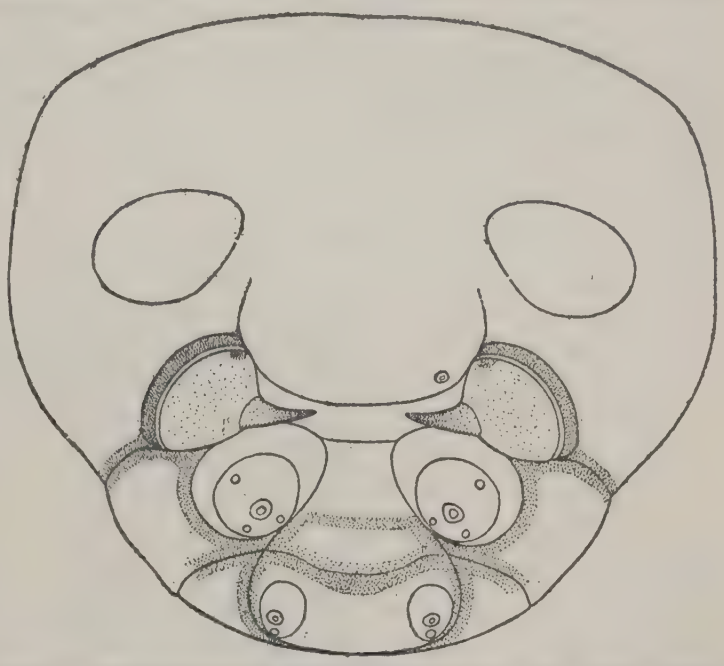

Fig. 14.—*Diachasma tryoni:* Head of mature larva, dorso-cephalic view. Greatest width 0.50 mm.

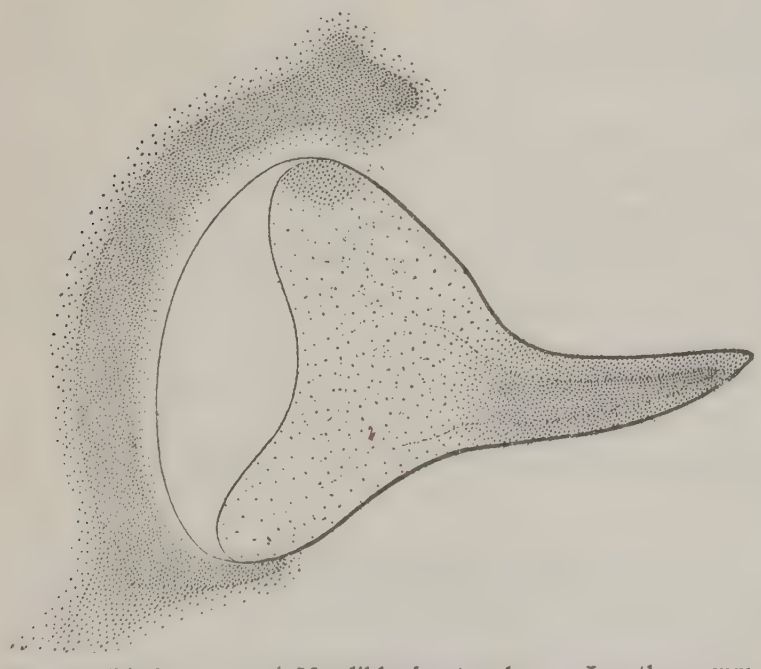

Fig. 15.—*Diachasma tryoni:* Mandible of mature larva. Length 0.12 mm.

sents fairly natural conditions. Table II shows the extent of hibernation occurring in the laboratory among fruit-fly puparia that were collected during every month throughout the year. The greatest hibernation occurred during the winter months commencing in December and the least during the warmest months. As the data are secured from material held in the laboratory in glass, the degree of hibernation is lower than would obtain in the field normally, which is evidenced by the box experiments.

TABLE II.—*Hibernation of Diachasma tryoni and D. fullawayi*[a]

Host puparia collected.	Total number of parasitized puparia.	Number of *D. tryoni* emerging in normal time.	Number of *D. fullawayi* emerging in normal time.	Number of Diachasma larvæ going into hibernation.	Total percentage of hibernation.
1916.					
December	302	193	10	99	32.8
1917.					
January	1,816	791	83	942	51.9
February	774	367	120	287	37.1
March	1,599	765	331	503	31.5
April	1,055	882	136	37	3.5
May	1,942	1,767	153	22	1.1
June	925	902	17	6	0.6
July	1,263	1,218	41	4	0.3
August	1,605	1,356	236	13	0.8
September	2,946	2,821	81	44	1.5
October	1,960	1,558	374	28	1.4
November	2,069	1,692	285	92	4.4
December	1,116	744	189	183	16.4

[a] The ratio of hibernating larvæ of *Diachasma tryoni* is greater than is that of *D. fullawayi*, but just how much greater as yet has not been determined.

The greatest emergences of adults from hibernating individuals occurs during the first seven months after the larva goes into hibernation. From 781 larvæ going into hibernation between August 1, 1916, and July 1, 1917, inclusive, 129, 119, 36, 67, 128, 147, 81, 27, 19, 16, 9, and 3 pupated and became adult during the period from the first to the twelfth month, respectively. As the greater number began their dormant period during the winter months and as the average duration of this period is from two to six months, it follows that the greatest emergence from hibernating individuals occurs in the spring and early summer months in Hawaii.

No doubt more than one factor enters into the cause of hibernation among the larvæ of the two species of Diachasma. Cool temperatures seem the most important, as suggested in Table II. During August, September, and October of 1917 the total hibernation, as occurring in material placed in glass vials, was 0.8, 1.5, and 1.4 per cent, respectively, based upon records of 1,605, 2,946, and 1,960 parasitized puparia, respectively. The average mean temperature at which this material was kept during the three months was 78.7°, 74.7°, and 75° F., respectively.

In the same months a quantity of parasitized, freshly formed fruit-fly puparia was placed in similar glass vials in a refrigerator running evenly from 60° to 64° during the entire duration of the experiment. From this material 384 adults of species of Diachasma emerged over a period somewhat retarded but not greatly prolonged, while 814 puparia failed to produce anything and upon being opened on December 20 were found to contain living, hibernating larvæ of this genus. This represents a hibernation of 67.7 per cent of all the material parasitized by species of Diachasma that was placed in the refrigerator, and is striking when compared with the hibernation of slightly over 1 per cent among the larvæ of this genus held at the same time at a temperature about 13 degrees higher. Another unknown cause for hibernation must exist, as material kept beneath sand or soil at any time of the year produces a greater degree of hibernation than does material held coincidently at nearly the same temperature but in dry glass vials exposed to light.

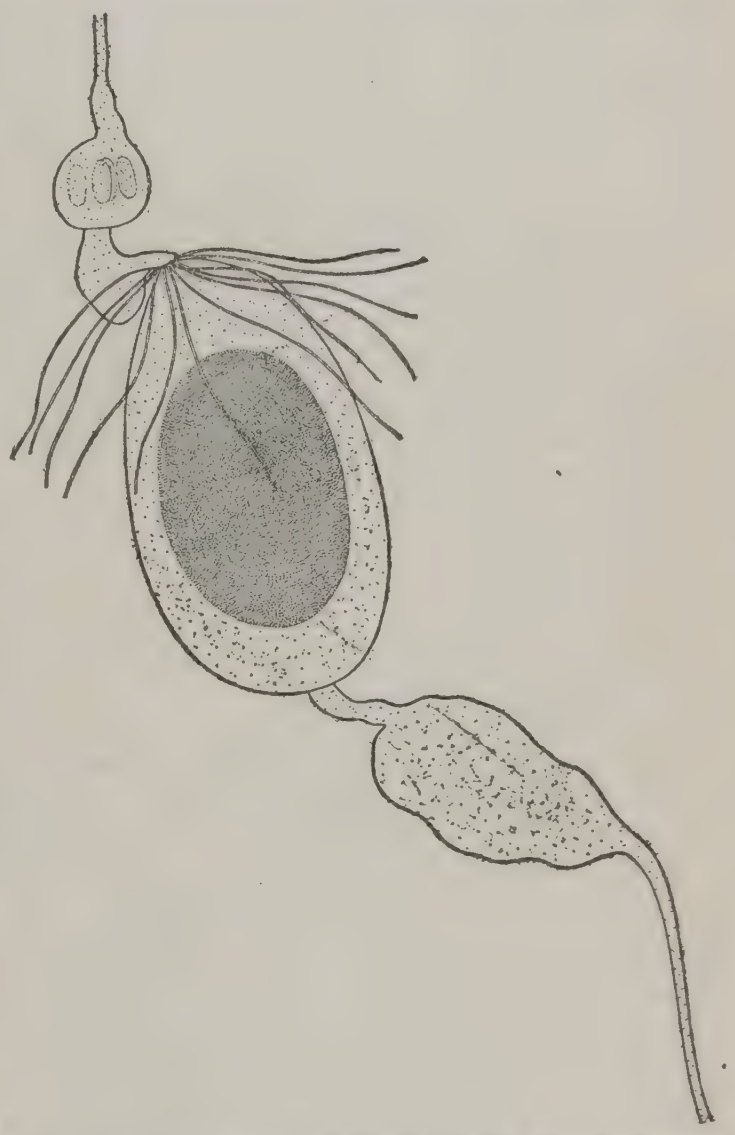

FIG. 16.—*Diachasma tryoni:* Alimentary canal removed from a mature pupa, showing the position and shape of the meconium. Greatly enlarged.

PUPA

The pupa is from 3.5 to 4 mm. long by 1.6 mm. wide and at first is pale white with reddish eyes. In a few days the adult colorations appear. At pupation the old larval skin is split from the head backwards and slips back to the caudal tip of the pupa and is there immediately pushed forward by the tips of the antennæ of the male or ovipositor of the female as these parts are forced forward over the dorsum of the body. The exuvium then comes to rest as a yellowish, crumpled mass at the tip of the ovipositor at a point generally over the pupal metathorax and extending, in part, back along the side of the ovipositor, or,

in the case of the male, it lies at the tips of the antennæ over the first two abdominal segments. When the adult parasite emerges, this exuvium is carried about for a short time on the antennæ of the male or the ovipositor of the female. With the complex changes in the alimentary canal, accompanying the formation of the pupa, the unvoided waste and accumulated food which fills the larval midintestine is forced to occupy a greatly reduced space. In the pupa, then, the midintestine is found to be short, oval, and filled with a dark compressed pellet (fig. 16). This pellet is the meconium. No portion of it is voided at the time of pupation or during the pupal period. In the mature pupa this meconium occasionally may pass into the hind intestine just caudad of the urinary tubes, but it never passes from the pupa. The pupa stage, following a short prepupal period of from 1 to 2 days, may last from 6 to 10 days. The duration of the pupa stage varies at any time of year.

The duration of the combined egg, larval, and pupal periods (Table III) is from 18½ days in midsummer to about 25 days in the coolest months. This represents the shortest period elapsing from egg to egg, for oviposition may occur on the day of emergence. This is a slightly shorter average period than obtains in the life of the fruit fly.

TABLE III.—*Duration of the combined egg, larval, and pupal stages of Diachasma tryoni and Opius humilis*

Diachasma tryoni.[a]					*Opius humilis.*				
Date.	Number individuals under observation.	Duration.		Mean temperatures.	Date.	Number individuals under observation.	Duration.		Mean temperatures.
		Extremes.	Average days.				Extremes.	Average days.	
1917				°F.	1916				°F.
Jan.	200	21–29	24.5	70.5	Jan.	34	18.5–23.5	20.5	71.0
Feb.	56	23–30	25	70.2	Feb.	177	17.5–25.5	20	72.6
Mar.	122	21–30	23.5	71.9	Mar.	83	17.5–23.5	19.5	73.2
1916					Apr.	208	16–21	17.9	74.2
Apr.	56	19.5–24.5	21	74.2	May.	258	15–21	17	75.2
May.	60	18.5–25.5	20	75.2	June.	46	13.5–17.5	15.5	75.6
June.	522	17.5–24.5	20	75.6	July.	42	13.5–16.5	14.5	76.8
July.	745	16–24	18.5	76.8	Aug.	104	14.5–17.5	15.5	76.6
Aug.	428	17–23	18.5	76.6	Sept.	133	14.5–17.5	15.5	77.3
Sept.	1,557	17–25	18.5	77.3	Oct.	100	15–19	16	76.0
Oct.	751	17.5–24.5	20	76.0	Nov.	158	15.5–20.5	17.5	75.0
Nov.	711	18.5–27.5	20.6	75.0	Dec.	104	18–23	19	72.4
Dec.	493	20–28	22.5	72.4					

[a] This table does not include hibernating individuals.

ADULT

The adult extricates itself from the host puparium by actively gnawing the part directly in contact with the head. In opening and closing the mandibles a transverse cut is made, usually in the third or fourth pupal segment and extending around about one-third of the circum-

ference. In pressing and working the head through this cut the entire end of the puparium usually is broken off and the parasite quickly emerges (fig. 17). In a few moments the antennæ, legs, and ovipositor straighten out. Immediately upon emerging the meconium is discharged. The period in the development of parasitic Hymenoptera when the meconium is discharged is interesting. With the opiines treated in this publication the meconium is never voided until the adult emerges. In the eulophid *Tetrastichus giffardianus* a major meconium is discharged just after the emergence of the adult and there is a barely perceptible discharge during the prepupal period. The pteromalid *Pachycrepoideus dubius* and the proctotrupid *Galesus silvestrii* void a large quantity of waste while in the prepupal stage and later, upon emergence, void an insignificant meconium.

Males nearly always commence emerging before the females and usually are all out while the females are still actively emerging. The period of greatest emergence of the males is from two to three days earlier than is that of the females. After leaving the puparium nothing can be found of the pupal skin. It is so extremely thin as to be almost invisible in water beneath a cover glass. Before the adult emerges, however, the pupal skin can be torn from the pupa as a thin, transparent covering.

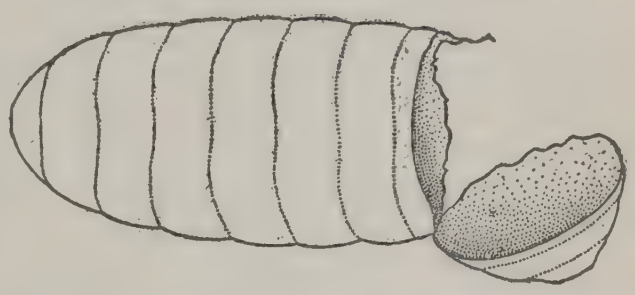

FIG. 17.—*Diachasma tryoni*: Fruit fly puparium showing emergence hole made by adult parasite. Length 4 mm. Typical of exit hole made by *D. fullawayi* and *Opius humilis*.

Copulation takes place most frequently during the first few days after emergence. In the laboratory mating has been repeatedly observed within 5 to 10 minutes after the adults have come out. The copulation period is short. It lasts from about 10 to about 60 seconds. The sex attraction is most strongly evident in the male. Practically all activity prior to and coincident with mating is on the part of the male. It becomes greatly excited when within 1 to 3 inches of the female and vibrates the wings rapidly and spasmodically. The male emits a strong, sweet odor. It is greatest in intensity in the presence of the female. No perceptible odor issues from the female. It never shows any great interest in the male and can readily repel it with the posterior pair of legs. In glass tubes the males make no distinction between mated and virgin females. Weak or injured females, unable to repel the male, may mate an indefinite number of times. A male may successfully copulate with different females more than once within a short period. A freshly emerged male was observed to mate with different females on August 24, 1917, at 10.20 a. m.,[1] 10.25 a. m., and 11.05 a. m. Normal

[1] All references to clock time relate to "standard time."

females confined with males in large sterilizing tubes have been observed in copulation as many as four to seven times during a given hour. Mating is probably best secured with the parasites confined in large screened cages a foot or more in diameter. Large cages with plenty of light are certainly superior to glass tubes for this purpose, although mating will occur in small shell vials. Under certain conditions males of *Diachasma tryoni* have mated with females of *D. fullawayi*. This may be brought about by confining many fresh males of *tryoni* with a few females of both *tryoni* and *fullawayi*. The progeny from two *fullawayi* females mated in this manner on June 29, 1916, were all males. Evidently these two species can not be crossed.

Males are more abundant than are females. During 1916 and 1917, 16,845 males and 10,130 females were bred from fruit-fly material collected in the field. Experiments conducted in the laboratory show that mated females produce a varying proportion of male and female progeny. From 11 mated females emerging on July 6 and 7 and August 24, 1917, and immediately isolated into separate cages for oviposition until death, the following progeny were secured: No. 1, 13 males and 23 females; No. 2, 24 males and 7 females; No. 3, 19 males and 27 females; No. 4, 5 males and 9 females; No. 5, 7 males and 21 females; No. 6, 14 males and no females; No. 7, 4 males and no females; No. 8, 49 males and 10 females; No. 9, 16 males and 15 females; No. 10, 23 males and no females; No. 11, 15 males and 2 females. In another case 25 freshly emerged females were placed separately with males on July 13, 1917, and left until all had mated. From these a total of 189 males and 86 females was reared. Three females, known to have mated with vigorous males four, five, and seven times, respectively, were given opportunity to oviposit from August 9, 1917, until death from three to four weeks later. From these a total of 47 males and 24 females was reared. The female which had mated seven times produced 14 males and 4 females. Such data indicate the importance of other factors than mere successful mating in the determination of sex proportion.

Unmated females of *Diachasma tryoni* as well as the other two opiine species treated herein are positively arrhenotokous. Ovipositing virgins during 1916 selected to prove this point consistently produced nothing but males.

Females may begin oviposition on the day of emergence, irrespective of whether they have mated or not. The ovaries are well filled with eggs, in a mature condition, at the time of emergence. Eggs are also present in varying degrees of development (fig. 19). A dissection of the ovaries of 24 females 24 hours old gave an average of 84 mature eggs per female. The greatest number of mature eggs found in a single day-old female was 125. Females which have been hibernating in the larva stage from 3 to 12 months are fertile, mate and reproduce, but are not

so prolific as are females coming from nonhibernating larvæ. The longer the larvæ have undergone hibernation, the weaker the reproductive system when the female finally emerges. An examination of the ovaries of nine females during 1916 and 1917, originating from larvæ hibernating from 3 to 12 months, gave an average of 37 mature eggs per female. Examination of the ovaries of two females originating as larvæ that had hibernated 12 months disclosed only 9 and 13 eggs, respectively. Examination of the ovaries of three females maturing from larvæ which had lain dormant for 10 months disclosed 31, 10, and 24 mature eggs, respectively.

OVIPOSITION

The maximum number of eggs usually are deposited during the first week of the life of the female. As many as 30 eggs have been deposited by a single female in a given day. A healthy female usually deposits from 5 to 9 eggs daily for the first week and only a few eggs daily thereafter. Death usually follows a few days after the cessation of egg-laying. The largest number of eggs deposited by a female in confinement was 148 (Table IV). This female lived only 12 days and died with 54 mature eggs in the ovaries. As noted later, ovipositing females do not live as long as do individuals given no opportunity to oviposit. Only one egg is deposited at a time. The total operation of laying a single egg requires from 15 to 45 seconds after the ovipositor has penetrated the fruit and located a host larva. A female may deposit an egg, rest a moment, and oviposit into the same or another larva immediately, but the ovipositor is completely removed after the placing of each egg. In ovipositing, the female moves about over the fruit, frequently pausing and moving in a circle over certain spots. The location of the larva, lying invisible in the fruit, appears to be wholly through a sense of touch. Judging from the actions of the female, all movements over the surface of the fruit indicate attempts to detect vibrations on the surface due to movements of the larva beneath the skin. When a larva is located, the female elevates the abdomen to an angle of about 45° and the ovipositor is brought to an almost vertical position with the tip resting against the fruit beneath the body. (Pl. 32, A.) The parasite never enters a broken fruit or penetrates into exposed pulp containing larvæ, all oviposition being entirely from the surface. Upon the insertion of the ovipositor the larva usually attempts to escape and frequently does. Barbs on the end of each of the two sharp, piercing ovipositor blades (fig. 18, *c*, *d*) probably enable the female to hold the larva in position, once the blades are fairly inserted. A third blade (fig. 18, *b*), which enters with the two piercing blades, but which is not sharp or barbed, is specifically designed for conveying the acid or poison from the acid and alkaline glands (fig. 19, *a*, *b*). The head end of this blade bears numerous perforations which are the surface openings of minute branching channels leading from the large central, hollow part of

this blade, through which the toxin flows during oviposition. A slight temporary paralysis of the larva seems to result after the ovipositor is fairly inserted, for little struggling ensues after the egg is extruded. The two other parts of the ovipositor, in addition to the piercing blades, consist of the outer pair of hollow sheaths (fig. 18, *a*, *e*), which do not penetrate beyond the surface of the fruit during oviposition unless the larva attacked lies under a large break or hole in the fruit. Several hours after

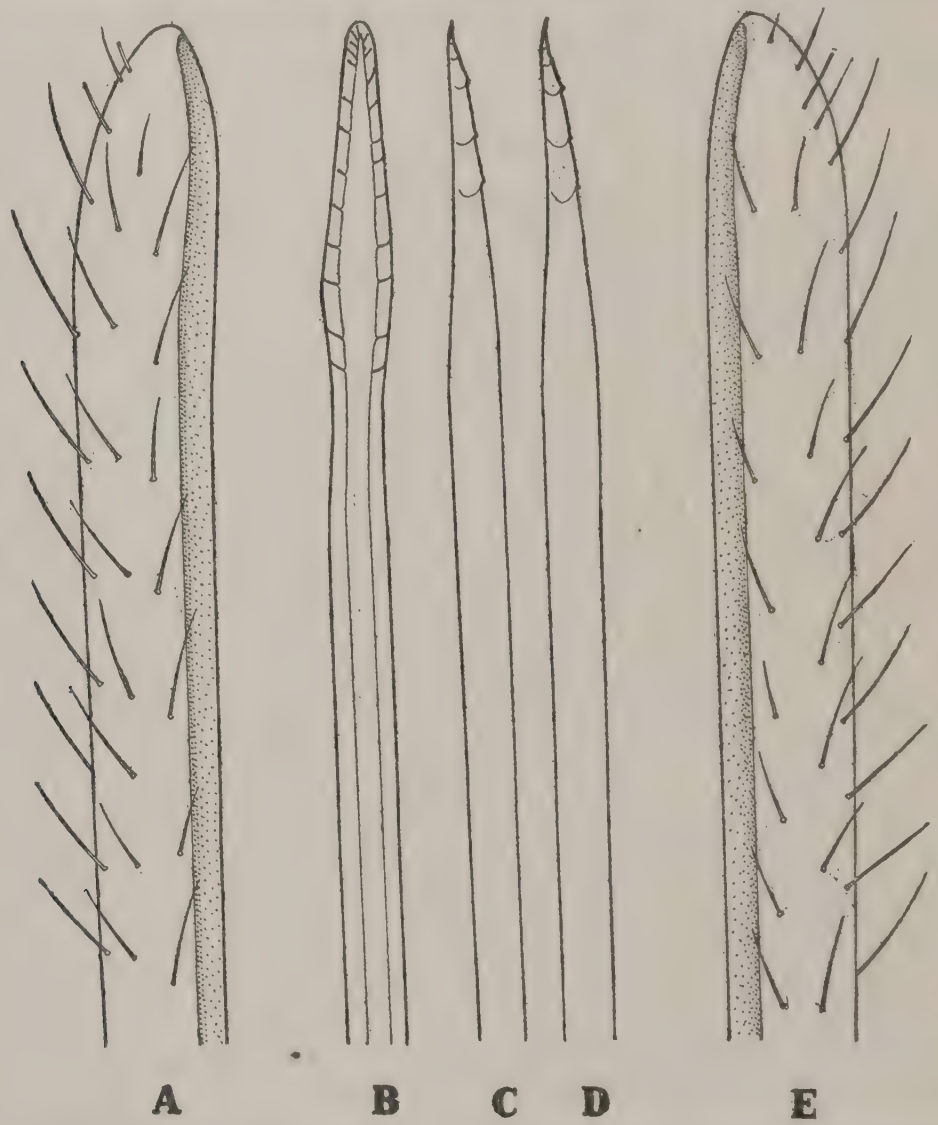

FIG. 18.—*Diachasma tryoni:* Parts of ovipositor: A, E, lateral sheaths; B, poison blade; C and D, piercing blades, showing characters of ends of each blade. Greatly enlarged.

a larva has been stung, a minute, oval, brownish spot develops on its body at the point where the ovipositor was inserted. This spot becomes a permanent scar and can be distinctly seen on the surface of the puparium. The female exhibits no decided capacity for discerning parasitized from unparasitized larvæ. Superparasitism is thus very common, although only one parasite ever develops in a superparasitized larva. This is owing to the cannibalistic habits of newly hatched larvæ of *Diachasma tryoni*.

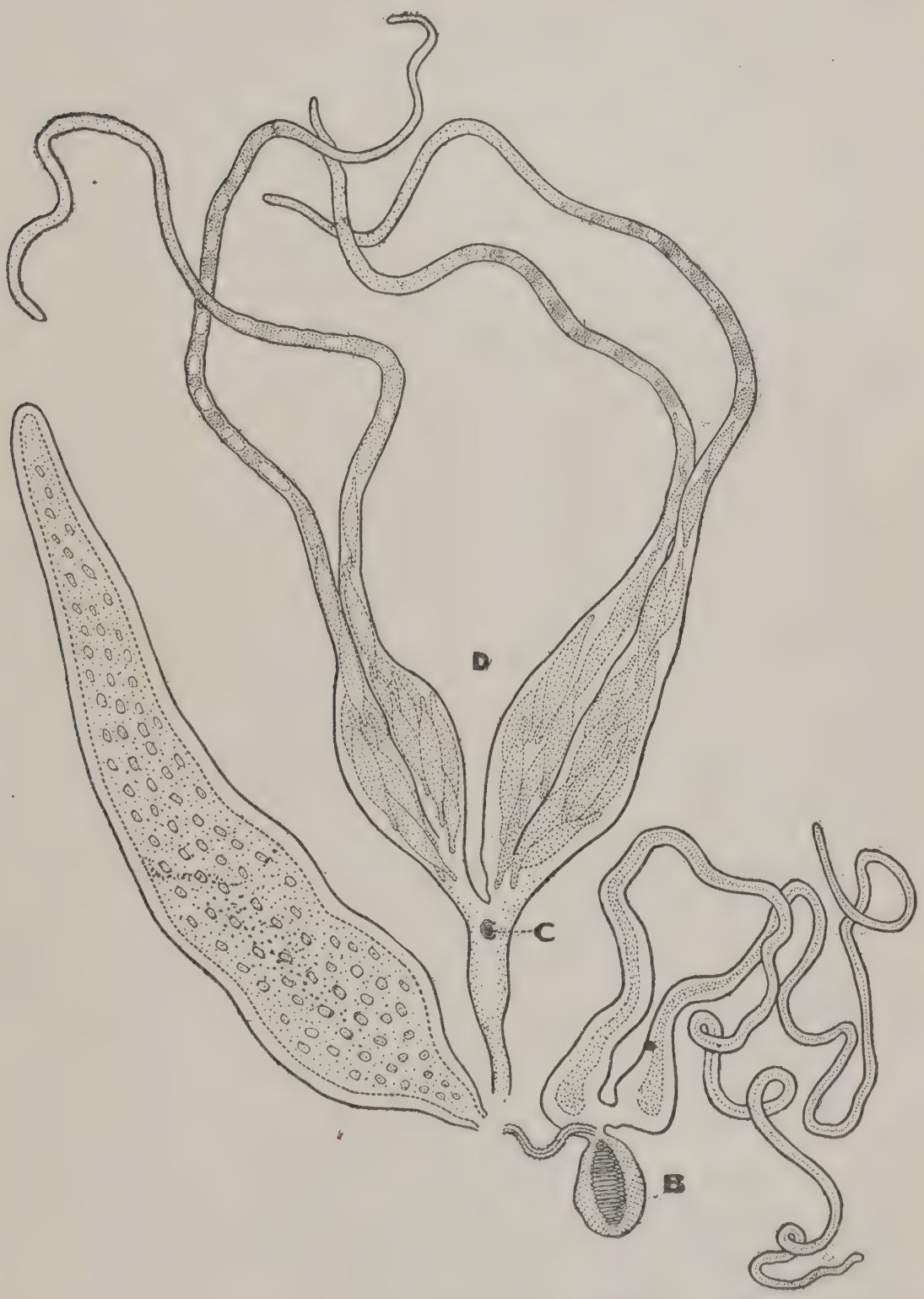

FIG. 19.—*Diachasma tryoni:* Reproductive system of newly emerged female: A, alkaline gland; B, poison reservoir with poison glands leading to it; C, spermatheca; D, ovaries, showing position and usual number of eggs and developing egg cells in newly emerged female. Greatly enlarged.

TABLE IV.—*Daily rate of oviposition of Diachasma tryoni, 1917*

Date of oviposition.	Number of eggs deposited.								
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.
May 18	.....	16	26	24	19	.....	.....	.....	.....
19	21	.....	11	12	3	.....	.....	.....	.....
20	22	6	17	5	15	.....	.....	.....	.....
21	8	16	.....	23	18	.....	.....	.....	.....
22	27	1	7	6	20	.....	.....	.....	.....
23	23	1	.....	.....	7	.....	.....	.....	.....
24	7	7	.....	7	30	.....	.....	.....	.....
25	1	4	.....	.....	14	.....	.....	.....	.....
26	.....	.....	.....	1	4	.....	.....	.....	.....
27	.....	.....	.....	Died	18	.....	.....	.....	.....
28	Died	Died	.....	.....	Died	.....	.....	.....	.....
June 5	.....	.....	Died	.....	.....	.....	.....	.....	.....
July 19	.....	.....	.....	.....	.....	3	.....	.....	.....
20	.....	.....	.....	.....	.....	5	2	.....	.....
21	.....	.....	.....	.....	.....	10	17	.....	.....
22	.....	.....	.....	.....	.....	8	13	.....	.....
23	.....	.....	.....	.....	.....	9	9	.....	.....
24	.....	.....	.....	.....	.....	13	21	.....	.....
25	.....	.....	.....	.....	.....	14	5	.....	.....
26	.....	.....	.....	.....	.....	10	.....	.....	.....
27	.....	.....	.....	.....	.....	9	5	.....	.....
28	.....	.....	.....	.....	.....	1	1	.....	.....
29	.....	.....	.....	.....	.....	4	.....	.....	.....
30	.....	.....	.....	.....	.....	10	1	.....	.....
31	.....	.....	.....	.....	.....	4	1	.....	.....
Aug. 2	.....	.....	.....	.....	.....	.....	Died	.....	.....
Oct. 9	.....	.....	.....	.....	.....	Died	.....	.....	.....
10	.....	.....	.....	.....	.....	.....	.....	2	.....
11	.....	.....	.....	.....	.....	.....	.....	6	12
12	.....	.....	.....	.....	.....	.....	.....	1	6
13	.....	.....	.....	.....	.....	.....	.....	15	15
14	.....	.....	.....	.....	.....	.....	.....	13	10
15	.....	.....	.....	.....	.....	.....	.....	12	8
16	.....	.....	.....	.....	.....	.....	.....	4	7
17	.....	.....	.....	.....	.....	.....	.....	7	7
18	.....	.....	.....	.....	.....	.....	.....	5	5
19	.....	.....	.....	.....	.....	.....	.....	7	4
20	.....	.....	.....	.....	.....	.....	.....	2	1
23	.....	.....	.....	.....	.....	.....	.....	.....	7
25	.....	.....	.....	.....	.....	.....	.....	.....	12
26	.....	.....	.....	.....	.....	.....	.....	Died	.....
27	.....	.....	.....	.....	.....	.....	.....	.....	2
28	.....	.....	.....	.....	.....	.....	.....	.....	6
30	.....	.....	.....	.....	.....	.....	.....	.....	1
31	.....	.....	.....	.....	.....	.....	.....	.....	1
Nov. 8	.....	.....	.....	.....	.....	.....	.....	.....	Died
Total.....	109	51	61	78	148	100	75	74	104

No. 1 to 5, inclusive, emerged on May 13 and were given fruit-fly larvæ in which to oviposit on May 18 and daily thereafter.

Nos. 6 and 7 emerged July 18 and Nos. 8 and 9 emerged on October 10.

No. 1 died with 10 mature eggs in the ovaries.
No. 2 died with 22 mature eggs in the ovaries.
No. 3 died with 0 mature eggs in the ovaries.
No. 4 died with 10 mature eggs in the ovaries.
No. 5 died with 54 mature eggs in the ovaries.
No. 6 died with 5 mature eggs in the ovaries.
No. 7 died with 38 mature eggs in the ovaries.
No. 8 died with 0 mature eggs in the ovaries.
No. 9 died with 42 mature eggs in the ovaries.

Although the mature fruit-fly larva is the stage most frequently attacked by the parasite, younger larvæ are often sucessfully parasitized. On September 15, 1916, a microscopical examination of the contents of 107 fruit-fly larvæ in the second instar, removed from fruit collected in Honolulu, gave interesting results. Twenty-four of these contained eggs or larvæ of either *Diachasma tryoni* or *Opius humilis*.

The female of *Diachasma tryoni* oviposits in larvæ in fruit after it has fallen to the ground and with equal facility attacks the larvæ in the fruit before it falls. The heaviest parasitism, however, probably occurs while the fruit is still on the tree. In March and April, 1917, a quantity of fruit, infested with fruit-fly larvæ known to be unparasitized, was placed on the ground beneath fruit trees and left for periods of 24 to 48 hours. It was then collected and placed in glass jars. From this fruit 208 individuals of *D. tryoni* were reared. A total parasitism by *D. tryoni* of 27.1 per cent was secured during August, September, and October, 1917, from 1,435 fruit-fly puparia coming from fruit collected from the trees in Honolulu. From 15,907 fruit-fly puparia secured from fruit collected from the ground during the same months a parasitism of 31.1 per cent by *D. tryoni* was obtained.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 13.3 per cent were parasitized by *Diachasma tryoni*. During 1917, as determined from collections of 72,139 larvæ, the parasitism by this species was 20.3 per cent.

Parasites confined in glass sterilizing tubes closed at one end, plugged with cotton, and kept continuously in partial darkness usually will remain alive and active for about two months. After eight or ten weeks of confinement the mortality is heavy. Only a few individuals have been kept alive beyond 80 days. Females not permitted to oviposit generally outlive the males. There is one record, however, of a male that lived for 125 days. Ovipositing females do not live in confinement much beyond 25 days even under the best of care. During May and June, 1917, 98 females were confined in small screened boxes kept in partial darkness and given material in which to oviposit constantly. Of these, 87 lived from 12 to 18 days and only 11 lived from 20 to 24 days. These were fed daily with a thin solution of honey and water placed in minute drops upon fresh leaves. Brown sugar, diluted with water in a ratio of 1 part of sugar to 5 of water, is a satisfactory food, though the results of feeding with diluted honey are better. A small portion of crushed apple or other fruit is relished by the parasites. Extract of beef added to the honey solution has been tried with unsatisfactory results. Concentrated honey or sugar solutions are also unsatisfactory. Parasites have been kept in a vigorous state longest when given honey diluted with 4 or 5 parts of water varied with a 3- or 4-hour period daily during which nothing but water is given.[1] Without food and held in bright light, the majority of the adults of this species under observation died in from 50 to 60 hours after emergence.

[1] For useful methods of confining parasites see PEMBERTON and WILLARD (4).

OPIUS HUMILIS

The parasite *Opius humilis* was brought to Honolulu from West Africa by Silvestri in May, 1913. It was soon established in the Kona coffee district of the Island of Hawaii, owing to the liberation there of a few individuals in June, 1913. By October of the following year it was found frequently parasitizing from 80 to 95 per cent of the larvæ developing in coffee in this district. Its general distribution and value were proven in Honolulu several months previously. As shown by data published elsewhere by the writers, the importance and effectiveness of this parasite soon became greatly curtailed through the restraint operated over it by the other introduced parasites.

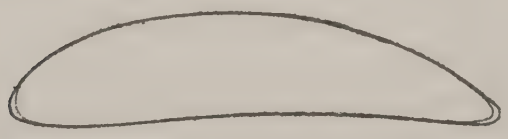

FIG. 20.—*Opius humilis*:: Egg freshly laid. Length 0.48 mm.

DESCRIPTION AND LIFE HISTORY

EGG

When first deposited (fig. 20), the egg is cylindrical, transparent, with smooth glistening surface, slightly curved and bluntly pointed at each end. The cephalic end is less pointed than is the opposite end. It is 0.48 mm. long and is nearly one-fifth as wide as long. No tubercular protuberances are present at either end when first laid nor is the outer enveloping membrane present that surrounds the egg of *Diachasma tryoni*. When fully developed it is 0.85 mm. long and less than one-third as broad as long (fig. 21), and is hardly recognizable as the egg deposited two days previously. Each end is prolonged into a distinct tubercle, the caudal end being prolonged much more than the opposite end. As in *D. tryoni*, the egg is placed just beneath the surface of the larval skin but so far as to be invisible from the surface. The wound made on the larva by the ovipositor remains permanently as an oval, brown scar.

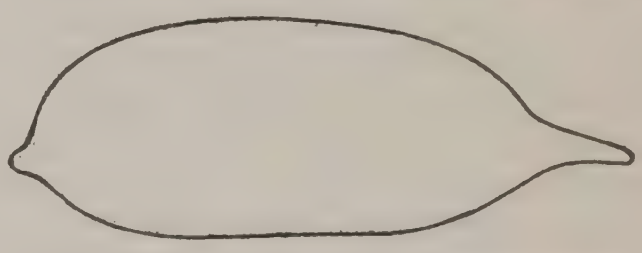

FIG. 21.—*Opius humilis:* Mature egg. Length 0.85 mm.

The duration of the egg stage ranges from about 45 hours in the summer months to 53 hours in the winter months. (For temperatures see Table III.) This is an average of about nine hours shorter than the similar period for *Diachasma tryoni*. Ten eggs deposited on March 24, 1917, at 10 a. m. hatched 48 hours later. One hundred and sixty-two eggs deposited on July 6, 1917, between 12 m. and 2 p. m. hatched from 45 to 47 hours later. Forty-two eggs deposited on May 13, 1916, between 10 a. m. and 12 m. hatched 48 hours later. The differences in duration are due to variations in temperature. The hatching of the egg and the effect of the egg upon the development of the host are identical with that of *D. tryoni*.

LARVA

The newly hatched larva (fig. 22, 23, 24) is almost exactly the same in size, structure, and habit as is the newly emerged larva of *Diachasma tryoni*, with the following exceptions: (1) The two pointed teeth situated at the middle of the cephalic edge of the chitinized ventral plate of the head are closer together than in *tryoni* and are joined basally to form a smoothly rounded letter "U"; (2) in *tryoni* the "U" formed by these two teeth is somewhat squarely made (compare fig. 3 and 24); (3) the head is somewhat smaller and squarer than is that of *tryoni;* (4) the ventral mass of serosal cells, retained after the hatching of the larva, is much smaller in volume, less conspicuous, and is often broken away from the larva before the latter molts to the second instar. The duration of this instar and the circumstances influencing the duration are almost identical with those of *D. tryoni*.

FIG. 22.—*Opius humilis:* Larva of first instar, lateral aspect, showing position and quantity of egg serosal cells clinging to ventral surface. Length 1.2 mm.

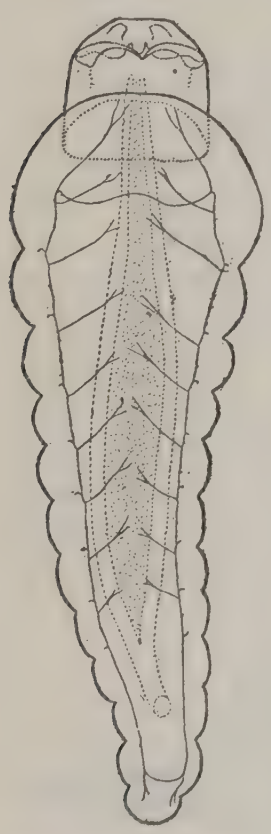

FIG. 23.—*Opius humilis:* Larva of first instar, dorsal aspect, showing head characters, complete tracheal system, and digestive canal. Length 1.1 mm.

The characters, habits, internal development, and duration of the second and third larval instars are so similar to those of *Diachasma tryoni* as to need no special comment. With the molt to the fourth instar, however, some distinguishing characters are readily seen. The mandibles are smaller than are those of *D. tryoni*, being 0.065 mm. long, more narrowly pointed, and wholly lacking in chitinization at the base (fig. 25). The dark chitinized ring at the base of the mandibles of the mature larva of *D. tryoni* immediately distinguishes it from larva of *Opius humilis* in the same instar. The period of the mature larva is short and does not extend much beyond five days. The larva never hibernates. As a large number of larvæ of species of Diachasma hibernate throughout the year, the absence of this trait in *O. humilis* renders it a more prolific parasite, in conjunction with its other characters, than is either of the two species of Diachasma. There are four larval instars.

PUPA

The pupa may be distinguished from the pupa of *Diachasma tryoni* by the short antennæ and ovipositor sheath. The ovipositor sheath extends beneath the body and up over the abdomen but the tips do not reach as far forward as the thorax. The pupa stage is slightly shorter than is that of *D. tryoni*.

FIG. 24.—*Opius humilis:* Molted skin of first-instar larva, showing head characters. Length 1 mm.

Although the egg, larva, and pupa stages are slightly shorter than are those of *D. tryoni*, the average period covering their combined development is consistently shorter by 3t o 4 days (Table III). During the summer months this combined period averages 15½ days and increases to about 20½ days in the winter. As eggs are deposited by the female on the day of emergence, the life cycle of this species covers a distinctly shorter average period than does that of its host.

ADULT

The emergence of the adult is similar to that of *Diachasma tryoni*. Males likewise precede the females by a day or more. The meconium is immediately discharged, as in *D. tryoni*. The pupal skin is extremely thin and difficult to see. The general habits of mating are identical with those of *D. tryoni*. The male emits a distinct but rather delicate sweet odor. No odor can be detected on the female. The proportion of sexes is better equalized than is the case with *D. tryoni*. During 1916 and 1917 a total of 6,128 males and 4,715 females was reared from material collected in the field. This is a percentage of 56.5 of males as compared with a percentage of 62.4 of males of *D. tryoni* secured over the same period.

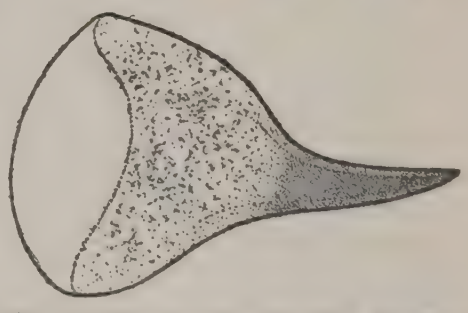

FIG. 25.—*Opius humilis:* Mandible of mature larva. Length 0.065 mm.

In confinement no conditions could be obtained under which this species would reproduce as favorable a proportion of the sexes as occurs in the field. The best results were obtained with females confined in a large glass jar (9 by 15 inches). In one experiment 25 males and 25 females were placed in such a jar immediately upon emergence, kept in strong light, and daily given material in which to oviposit. In three days' time mating and oviposition oc-

curred, resulting in the rearing of 114 males and 52 females. It has been found possible to rear reasonable quantities of females under various conditions of confinement. A large, well-lighted glass or screen cage is strongly recommended. Cages less than 9 or 10 inches in diameter, or even small glass tubes, can always be safely used for ovipositing females, but satisfactory mating does not occur in small cages. From 100 males and 75 females placed, upon emergence, in a small cage having a diameter of 2½ inches and a length of 7 inches, a total of 558 males and 27 females was reared during their lifetime. This is an average result in rearing this species when the cage is small. It shows an abundant oviposition but little mating.

Unmated females reproduce parthenogenetically, the progeny being always males. These males have been proved fertile.

OVIPOSITION

Oviposition may commence upon the day of emergence. An average of 80 mature eggs occurs in the ovaries of the newly emerged female. The manner of egg deposition is similar to that described for *Diachasma tryoni*. Only one egg is deposited at a time. An average period of 17 seconds is required for the deposition of a single egg, after the ovipositor has penetrated the fruit and located a larva. This is based upon the timing of 31 separate egg depositions. The shortest time was 10 seconds and the longest 3 minutes and 15 seconds. The female attacks larvæ in fruit on the ground as well as larvæ in fruit on the tree, she may oviposit into fruit-fly larvæ in the second instar, and selects no particular part of a larva in which to oviposit. The daily rate of oviposition is indicated in Table V. Female 6 therein is of unusual interest. A total of 255 eggs, deposited quite generally over a period of 20 days, indicates that this species oviposits probably over a longer period than does *D. tryoni*. The greatest number of eggs deposited in 24 hours was 34. It is of interest that most of the individuals shown in Table V died shortly after the last egg was deposited. Female 6 was given, in addition to honey and water in the proportions of 1 part honey to 6 of water, a daily feeding of pure water. During the morning hours nothing but water was given, the honey being added in the afternoon. All of the parasites used in the oviposition records were confined in glass cylinders 6 inches long and 1 inch in diameter, open at both ends but protected with screened caps. Unparasitized fruit-fly larvæ reared in the laboratory were used in obtaining the records. The larvæ were daily placed in the fruits of *Mimusops elengi* (Plate 32, A) and were removed daily thereafter and dissected, under magnification, for eggs of the parasite and replaced daily by others so that the experiment might be continued until the death of the females.

TABLE V.—*Daily rate of oviposition of Opius humilis. 1916–17*

Date of oviposition.	Number of eggs deposited.					
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Oct. 10	6					
11						
12	17					
13	11					
14	18					
15	23					
16	16					
17	8					
18	5					
20	1					
25	1					
Nov. 1	Died.					
July 6		2	2	3	3	
7		14	10	6	5	
8		2	7	2		
9		32	1	10		
10		7	1	5		
11		5	1		4	
12		15	14	19	10	
13		19	8	1	8	
14			4	5	1	
15					1	
16		28		11		
17		9	4			
18			11			
19		4	5	5		
20		4	18	12		
21			6	12		
22				5		
23			3			
24		1	5	3		
25			6			
26			4	1		
27						18
28						24
29						24
30			Died.	Died.		10
31						25
Aug. 1						34
2						22
3						16
4						10
5					Died.	2
6						13
7		Died.				8
8						6
9						6
10						8
11						5
12						4
13						11
15						9
23						Died.
Total	109	142	110	100	32	255

No. 1 emerged on October 10, 1916.
Nos. 2, 3, 4, and 5 emerged on July 6, 1917.
No. 6 emerged on July 27, 1917.
No. 1 died with 14 mature eggs in the ovaries.
No. 2 died with 8 mature eggs in the ovaries.
No. 4 died with 18 mature eggs in the ovaries.
No. 6 died with 10 mature eggs in the ovaries.
No examination was made of the ovaries of Nos. 3 and 5 at death.

Superparasitism in the field is common and indiscriminate, no preference for parasitized or unparasitized larvæ being evident.

The ovipositor blades of this species, though hardly one-third as long as those of *Diachasma tryoni*, are otherwise almost identical in structure, position, and number.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 17.2 per cent were parasitized by *Opius humilis* and during 1917, as determined from collections of 72,139 larvæ, parasitism by this species was 12.7 per cent.

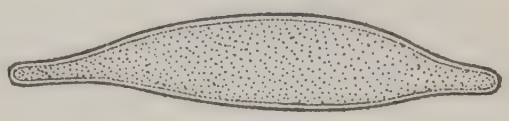

FIG. 26.—*Diachasma fullawayi:* Freshly deposited egg. Length 0.33 mm.

Individuals held in glass sterilizing tubes and kept in partial darkness lived somewhat longer than did adults of *D. tryoni*, when fed and held under the same conditions. Of 541 individuals confined with diluted honey and water as food, 18 females lived 100 days or more, two of these living until 125 days old.

DIACHASMA FULLAWAYI

Diachasma fullawayi was brought to Honolulu by D. T. Fullaway in October, 1914. The material was secured in West Africa by D. T. Fullaway and J. C. Bridwell. It was originally found in West Africa by Silvestri in 1912.

DESCRIPTION AND LIFE HISTORY

EGG

When first deposited, the egg (fig. 26) is about 0.33 mm. long and 0.066 mm. wide. At maturity (fig. 27) it is about 0.66 mm. long and 0.22 mm. wide. Other than being slightly broader than is the egg of *D. tryoni*, it is almost identical with the latter in development, shape, color, size, duration of stage, and manner of hatching.

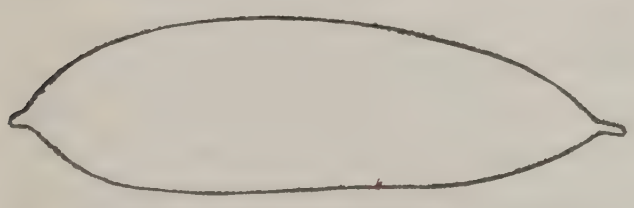

FIG. 27.—*Diachasma fullawayi:* Mature egg. Length 0.66 mm.

LARVA

The newly hatched larva has been found to differ from that of *D. tryoni* in one noticeable respect. The cephalic edge of the chitinized ventral portion of the head bears three pointed teeth instead of two, as in *D. tryoni*. The middle tooth is less than one-half as long as the other two (fig. 28). The color of the chitin in the head is a shade darker than in *D. tryoni*. Otherwise the two larvæ are practically identical in appearance, movements, internal structure, feeding habits, and duration of the instar.

The larvæ of the second and third instars have not been distinguished in any respect from those of *D. tryoni*. The mature larva, which is the fourth instar, differs from the *tryoni* larva only in being faintly yellowish in color and in having a slightly darker chitinization of the mandibles (fig. 29). The mature larva of *D. fullawayi* also hibernates, but the percentage of larvæ hibernating appears to be less than occurs with *D. tryoni;* the period of hibernation also is shorter. The greatest amount of adult emergence from hibernating individuals occurs during the first three months after the larva matures. From 68 larvæ passing into a state of dormancy between August 1, 1916, and July 31, 1917, inclusive, 29, 16, 21, and 1 pupated and became adults during the period from the first to the fourth months, respectively. One individual hibernated for 8 months and 15 days. The greater proportion of the larvæ under observation went into hibernation during the months of November, December, January, February, and March, and the greatest period of adult emergence from hibernating material was in March, April, and May. A much greater degree of hibernation occurs in fruit-fly puparia left in soil or sand than obtains in dry glass vials.

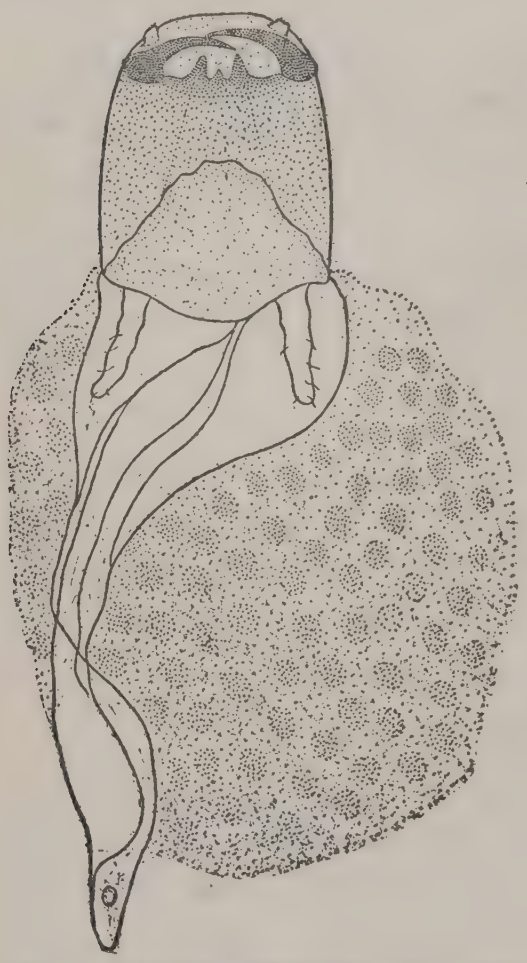

FIG. 28.—*Diachasma fullawayi:* Cast skin of first-instar larva, showing head characters and egg serosal cells still clinging to ventral surface. Length 1 mm.

PUPA.

The pupa may be distinguished from that of *D. tryoni* by the unusually long ovipositor sheath which extends back over the body almost to the head. The duration of the pupa stage is the same as that of *D. tryoni*, as is also the duration of the combined egg, larva, and pupa stages.

ADULT

The behavior of *D. fullawayi* after general distribution in Hawaii is interesting. Localities having high humidity and precipitation have proven especially favorable for this species. At points where the average humidity is low it has had evident difficulty in existing at all, even under very favorable host conditions. An investigation of this species in 1917, after wide distribution and thorough establishment, clearly indicated the particular capacity of this species for life under

humid conditions. Of 2,232 fruit-fly puparia collected in the Kona coffee district of the island of Hawaii in 1917, 2 per cent were parasitized by *D. fullawayi* and the percentage of parasitism here, where the precipitation averages about 50 inches a year, was about the same in 1916. On the opposite side of the island, about the town of Hilo, where the precipitation averaged 200 inches during 1916, parasitism by this species reached 60 per cent, as determined from 316 fruit-fly puparia collected from coffee. During 1917 an unprecedented drought was experienced on the Hilo side of the island and parasitism by *D. fullawayi* was reduced to a fraction of 1 per cent. From 700 fruit-fly puparia secured from that source during November, 1917, at the time of the drought, no individuals of *D. fullawayi* were reared, although the other opiines were abundant, particularly *Opius humilis*. Fifteen miles from Hilo, however, in a locality more elevated and forested, where the humidity can not get very low, the parasitism by *D. fullawayi* was 98.2 per cent, as determined from a collection of 259 fruit-fly puparia secured from coffee on the same date as that on which the Hilo collection was made. Perhaps the most significant evidence that can be given to bear out this point is the parasitism by this species during 1917 in upper Manoa Valley in Honolulu and at the Maunawili Ranch, Oahu. At these two localities the precipitation averages 150 inches a year and at Maunawili the Weather Bureau records show an average of 324 rainy days a year. From 78 fruit-fly puparia collected at Maunawili during 1916 the parasitism by *D. fullawayi* was 65.3 per cent. From 1,542 puparia secured at that place in 1917 the parasitism by this species was 88.4 per cent. From 474 puparia secured from coffee in upper Manoa Valley in 1917 the parasitism by *D. fullawayi* was 91 per cent. Honolulu, on Oahu, has a precipitation closely paralleling that of the Kona district on the island of Hawaii, usually averaging 50 inches a year. In Honolulu the average collections of fruit produce fruit-fly larvæ only slightly parasitized by *D. fullawayi*, even though upper Manoa Valley is only a few miles removed from Honolulu. Still the difference in rainfall is great between the two localities.

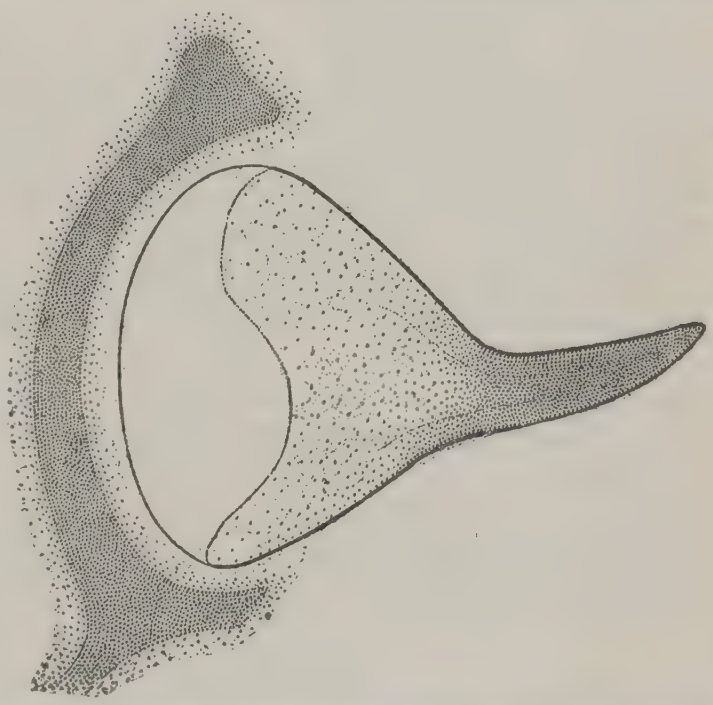

FIG. 29.—*Diachasma fullawayi:* Mandible of mature larva. Length 0.12 mm.

In addition to being especially adapted for propagation in wet areas, this parasite, for some unexplained reason, favors certain fruits harboring the host larvæ. It is produced in Honolulu most frequently from fly puparia secured from the loquat (*Eriobotrya japonica*), bestill (*Thevetia neriifolia*), French cherry (*Eugenia uniflora*), coffee, and the fruits of *Chrysophyllum monopyrenum*. It will frequently attack larvæ in these fruits to the almost total exclusion of heavily infested fruits of the kamani that may be growing close by. It particularly favors the loquat.

Emergence, mating, and oviposition are not different, so far as can be noted, from these habits as described for *D. tryoni*. No odor is emitted by the male or female. A meconium is discharged immediately after emergence. The females may reproduce parthenogenetically, the progeny being always males.

Adults of this species have been kept alive longer than have any of the other parasites. Between December, 1916, and May, 1917, of 235 adults held in confinement in glass tubes and fed honey and water 43 lived over 100 days, 11 lived 120 days or over, 1 lived for 134 days, and 1 for 141 days. Most of the long-lived individuals were females. Three males lived for 120, 121, and 127 days, respectively. Without food and held in bright light the majority of the adults die in about 55 hours. One male has been kept alive for 103 hours and 2 females for 79 hours.

The proportion of sexes is more favorable in this species than in the case of either *D. tryoni* or *Opius humilis*. During 1916 and 1917 a total of 5,528 males and 5,566 females emerged from fruit-fly material collected in the field.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 2.1 per cent were parasitized by *D. fullawayi*. During 1917, as determined from collections of 72,139 larvæ, the parasitism by this species was 7.3 per cent.

TETRASTICHUS GIFFARDIANUS

This species was brought to Honolulu from West Africa by Fullaway in October, 1914. It was collected and colonized in Africa by Fullaway and Bridwell. It soon became largely propagated and widely distributed in the Territory of Hawaii. Though its life cycle is short and its development as rapid as is that of the opiines, its importance as a parasite prior to 1917 was doubtful. During 1917, however, it had become better established and had increased very considerably the total parasitism of the fruit fly about Honolulu in fruits having thick pulps, such as oranges, peaches, and guavas. Larvæ in these fruits are not easily reached by braconid parasites. It is the most prolific of any of the introduced parasites of the fruit fly, and, under favorable host and weather conditions, may multiply enormously in localized spots. Owing to the short life of the adult, the absence of a hibernating form, its small size, and seemingly poor powers of flight, it rapidly drops off in effectiveness as soon as host material becomes scarce within a short radius.

DESCRIPTION AND LIFE HISTORY

EGG

The newly deposited egg measures from 0.20 to 0.25 mm. in length, is less than a third as broad as long, is pale white, smooth, glistening, cylindrical, and broadly rounded at each end (fig. 30). The eggs are placed in clusters just beneath the larval integument. They are faintly visible from the surface as dark bodies if strong light is transmitted through the larva from below. The egg does not undergo much change in size as the development of the embryo progresses. The duration of the egg stage is from 2 to 2½ days. Of 400 eggs deposited January 4, 1917, at 11 a. m., all hatched in about 60 hours. Fruit-fly larvæ containing eggs of this parasite develop to maturity and form a perfect puparium, in the same manner as do the other parasites under discussion. No fly pupa is formed after the histolysis of the larval tissues in the puparium, and the egg may hatch either before or after the puparium is formed.

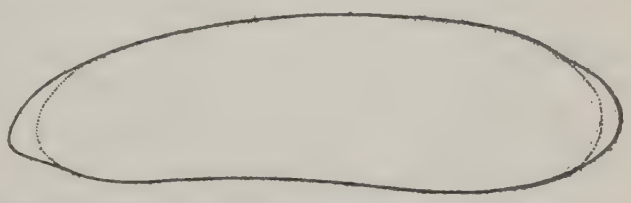

FIG. 30.—*Tetrastichus giffardianus:* Egg newly deposited. Length 0.25 mm.

LARVA

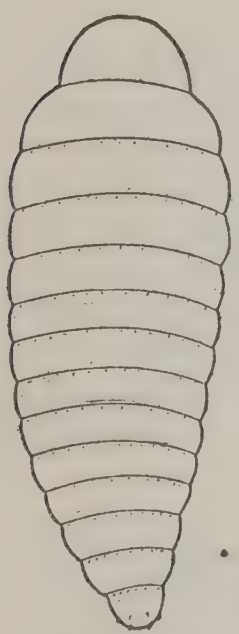

FIG. 31.—*Tetrastichus giffardianus:* Newly hatched larva. Length 0.25 mm.

There is nothing of unusual interest in the development of the larva, and only little external difference in the appearance of the instars, with the exception of the gradual increase in size. When first hatched, the larva is about 0.25 mm. long (fig. 31). The body is composed of 13 segments and head, the latter inconspicuous and not unlike the other body segments in general appearance. The mandibles are minute, short, curved, broad at the base, and well chitinized. With only slight modification they are constant in the succeeding instars. Most of the food ingested by this parasitic larva consists of fat bodies. The larva bears 9 pairs of small open spiracles on body segments 2 to 10, inclusive. The tracheal system is open and becomes air filled as soon as the larva hatches. In the first instar the trunks and branches are very faint and threadlike. The larva can move only very slowly and sluggishly in all of its instars.

The number of larval instars has not been determined, though three forms have been distinguished. The mature larva may be distinguished readily by the large, heavy tracheæ and stigmata. As 8 or 10 larvæ nearly always develop in a single host individual, and the food is rapidly consumed, the necessity for well-developed tracheæ is thus apparent

even within a few days after the larva hatches. No cannibalism occurs among the larvæ of this parasite although from 20 to 30 individuals may be developing simultaneously. In such congested cases they nearly all mature, but result in dwarfed adults. No waste matter is passed by the larva, the midintestine being closed caudally during the entire period of larval development. The larva enters a short prepupal period, from 2 to 3 days in duration, before pupating. During this prepupal period a very small portion of the accumulated waste is voided. This would indicate that the mesenteron and proctodeum become connected even before the pupa is actually formed. No hibernating form of the larva is known to occur.

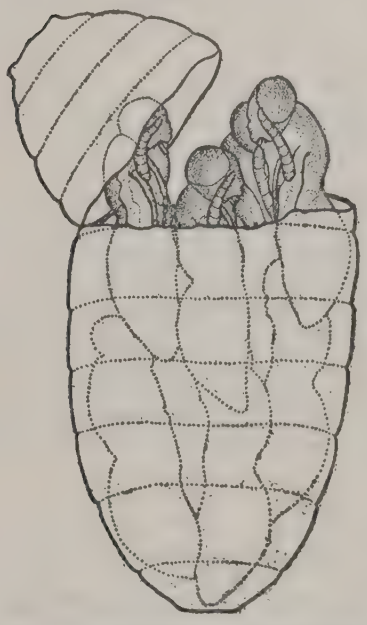

FIG. 32.—*Tetrastichus giffardianus:* Pupæ in normal position and number in fruit-fly puparium opened to show contents. Greatly enlarged.

The pupa is formed with its head facing the cephalic end of the puparium (fig. 32). In cases where 20 or 30 pupæ are packed within the puparium, a few may lie with the position reversed. Of 200 puparia containing pupæ of *T. giffardianus*, examined during August and September, 1917, 3 per cent of the pupæ were lying with the head in this reversed position.

The duration of the combined egg, larva, and pupa stages is from 24 to 31 days in the cool months and lasts about 18 days during the warm months. In January, 1917, 243 adults emerged in from 24 to 31 days after the eggs were deposited, with an average period of 28 days. In April, 1917, 894 adults emerged in from 19 to 26 days after the eggs were deposited, with an average period of 21 days, and in July, 1917, 455 adults emerged in from 17 to 19 days after the eggs were deposited.

ADULT

The adult emerges from the puparium by gnawing a small hole, more or less ragged and circular. Though the puparium may contain from 3 to 30 parasites, usually only one emergence hole is made. The typical position of the emergence opening is shown in figure 33. Occasionally two holes are made, and rarely three. They may be at either end of the puparium or between the extremities. More than one emergence hole usually results from the development of an excessive number of individuals in the puparium. In one rare case wherein 39 parasites emerged from a single puparium, three emergence holes were made. A distinct, thin, brownish pupal skin is left in the puparium after the adult has issued. Although wrinkled and twisted, the skin is an exact replica of the mature pupa. The parasites may twist and turn about

in the puparium for many hours before an opening is cut through which they can escape. Males and females all appear to emerge at the same time, for as soon as a sufficiently large exit hole is made, the adult flies come out as quickly as possible, irrespective of sex. The males remain hovering about the puparia but the females immediately crawl away. The meconium, which is developed and retained in the mid-intestine by the larva and held there in the pupa stage, is voided by the adult immediately upon emergence.

Mating occurs as soon as the adults are out of the puparium. The entire process occupies only a few seconds. Females once mated have no difficulty in warding off the males. No mating occurs within the puparium even though the parasites may be actively moving about in it for several hours before they escape. This is proved from the fact that all females taken immediately after emergence and isolated produce parthenogenetic males. One male may mate with several females within a few hours. On September 8, 1916, one male was placed with 8 virgin females and left for four days. Each female was then placed in a separate vial and given opportunity to oviposit. All produced both male and female progeny. That the male is particularly capacitated for frequent mating is to be expected when the proportion of the sexes is considered, the females always greatly outnumbering the males. During 1916 and 1917 a total of 13,114 males and 47,804 females, or 78.5 per cent, emerged from fruit-fly puparia collected in the field about Honolulu.

FIG. 33.—*Tetrastichus giffardianus:* Fruit-fly puparium showing characteristic emergence hole made by adult parasite.

Unmated females are always arrhenotokous. From the proportion of sexes secured from field collections, it is shown that the smaller proportion of males readily gain access to and mate with the much larger proportion of females.

OVIPOSITION

Females may begin ovipositing as soon as they emerge, whether mated or not. The mature pupa has well-developed eggs in the ovaries. Fourteen newly emerged females when examined October 20, 1917, contained 73, 70, 72, 60, 73, 66, 71, 81, 65, 45, 58, 64, 61, and 52 mature eggs, respectively. The method of oviposition is best understood by an examination of Plate 32, B, C, and D. The female enters the fruit wherever access can be gained through holes, decayed spots, or breaks on the surface. There is no evidence to indicate that the parasite bores into firm pulp or into the skin of the fruit. Once into the fruit, however, the female may become attached to a larva and be drawn through all manner of pulp and juice before her object has been attained. As soon as a larva is located, the ovipositor is quickly brought forward and beneath the

body and inserted into the body of the larva. In this operation the body is reared up with the front pair of legs stretched at full length or often drawn free from the larva. Only one or two seconds are required for the insertion of the ovipositor. The barbs at the tips of the piercing blades now hold the female securely to the larva. By the time the ovipositor is well inserted the female is standing straight up on the ovipositor and balancing, often on only one posterior leg and the ovipositor or on the ovipositor alone, as shown in the photomicrographs (Pl. 32), after which the female moves very little. The legs and wings are held perfectly still, although the larva may frantically bore about in the pulp or escape entirely from the fruit, in an endeavor to dislodge the parasite. To all contortions the female parasite remains indifferent. When the larva is held in position beneath a microscope a rapid transference of liquid and eggs can be seen passing down the ovipositor. After the eggs are inserted the ovipositor is withdrawn and sheathed in from 2 to 3 seconds. The ovipositor is not removed until the female brings all feet down again in touch with the larva or anything that may be conveniently in reach. The female deposits an average of 8 eggs in a cluster, with each insertion of the ovipositor. Between September 4 and 21, 1916, 50 separate ovipositions were under careful observation. The time elapsing for each oviposition ranged from 9 to 60 seconds, with an average of 35.6 seconds. The number of eggs deposited at each oviposition ranged from 2 to 16 with an average of 8.6. This average is interesting as a laboratory experiment, for it was found that from 3,527 adults of *T. giffardianus* emerging from 412 puparia collected in the field during 1916 and 1917, the average number emerging from these puparia was 8.6 per puparium. It indicates that the parasite generally oviposits only once into the same larva and that the larva is not again stung by another individual, in the majority of cases. One curious point in the rearing of this species from field material should be emphasized. In every case under actual observation wherein a fly puparium produced several females of *T. giffardianus*, one male, and occasionally more than one, developed in the same puparium with them. In other words, each time a mated female places a cluster of 6 to 8 eggs or more in a larva, at least one of those eggs is destined to produce a male, for usually 1 male and 6 or 7 females ultimately emerge.

It has been assumed generally that this parasite works mostly about the ground. Certain evidence has been secured to show that it works a great deal above ground also. Of 94 fruit-fly larvæ emerging from bestill fruits (*Thevetia neriifolia*) picked from trees on August 28, 1917, 18.1 per cent were parasitized by *T. giffardianus*. Of 192 larvæ secured from the same host picked from the trees on August 16, 1917, 9.9 per cent were parasitized by *T. giffardianus*. Forty-five larvæ from the same kind of fruit picked from the trees on September 4, 1917, produced 13.3 per cent *T. giffardianus*. Of 533 fruits of the kamani picked from trees

on October 8, 1917, 7.3 per cent were parasitized by *T. giffardianus*. Parasitism by *T. giffardianus* of larvæ from these fruits, and other varieties picked from the ground on the same dates, was not appreciably higher than that just cited. The individuals reared from larvæ coming from kamani fruits picked from the trees indicate that the females may fly well up above the ground, for the lowest fruits of the kamani are usually from 10 to 12 feet above the ground. It is a high, wide-spreading tree.

Although the female oviposits into any portion of the host larva within reach, the majority of the eggs are placed in the posterior half of the body. This is because the larva buries its head into pulp or juice almost as soon as a female touches it, and, in her attempts to cling to the larva and insert the ovipositor, the attachment is not ordinarily made until she has been drawn back toward the posterior end. The female may oviposit into a larva without being actually in contact with it. This occurs when the ovipositor is forced through thin layers of pulp under which the female may detect the presence of a larva. The larva must be close to the female, however. A distinct, brownish, oval scar remains on the larva at the point at which the ovipositor has been inserted. This character is more clearly discernible after the larva has formed into a puparium.

The maximum number of eggs deposited by a given female in confinement was 104 (see Table VI). The greater number of eggs is deposited during the first five or six days after the female emerges. They are usually deposited in large lots at intervals 24 to 48 hours apart, rather than in small numbers frequently. The greatest number of eggs deposited by a given female over a period of 24 hours was 62. No ovipositing female has been kept alive longer than 12 days. The great prolificness of this species is here seen from its habit of oviposition and in the proportion of sexes above mentioned. With an average life cycle of about 25 days, a deposition of from 90 to 100 eggs within the first five or six days of life, and the resulting progeny 75 per cent female, the multiplication is seen to be very rapid. Fruit-fly larvæ in the second instar may be successfully parasitized.

Adults fed with honey and water and confined in glass tubes held in partial darkness have been kept alive from two to three months without difficulty, provided no opportunity is given for oviposition. Of 274 adults emerging on September 3, 1917, one male lived for 61 days and one female for 69 days. Most of the males died between the ages of 30 and 50 days, while the majority of the females died between the ages of 40 and 60 days. Of 267 adults emerging on December 19, 1917, one male lived for 58 days and one female for 102 days. Nine of the females lived over 90 days. This long life was due to the mild temperatures of December, January, and February. Without food this parasite usually dies within 46 hours, although one individual has been held alive for 55 hours.

TABLE VI.—*Daily rate of oviposition of Tetrastichus giffardianus, 1916–17*

Date of oviposition.	Number of eggs deposited.														
	Female No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
October.															
9	30	....	34	....	....	....	....	....	....	....	....	....	....	....	....
10	....	53	....	....	10	....	9	12	8	7	14	....	....	....	....
11	12	6	30	51	24	52	20	7	....	3	1	....	....	....	....
12	22	7	....	23	(a)	7	4	7	7	....	10	....	....	....	....
13	....	....	....	....	....	....	....	21	10	8	1	....	....	....	....
14	10	8	....	....	....	....	13	4	15	....	4	....	....	....	....
15	....	....	....	10	....	20	....	1	....	10	....	....	....	....	....
16	....	15	9	....	....	....	....	(a)	8	8	....	....	....	....	....
17	(a)	....	12	....	....	(a)	....	....	....	5	(a)	....	....	....	....
18	....	....	(a)	(a)	....	....	....	....	....	....	....	....	....	....	....
20	....	15	....	....	....	....	....	....	....	(a)	....	....	....	....	....
21	....	(a)	....	....	....	....	....	....	....	....	....	....	....	....	....
23	....	....	....	....	....	....	(a)	....	(a)	....	....	....	....	....	....
July.															
27	....	....	....	....	....	....	....	....	....	....	....	....	2	24	61
29	....	....	....	....	....	....	....	....	....	....	....	....	53	24	....
30	....	....	....	....	....	....	....	....	....	....	....	....	....	....	7
31	....	....	....	....	....	....	....	....	....	....	....	62	7	10	....
August.															
1	....	....	....	....	....	....	....	....	....	....	....	....	(a)	....	25
2	....	....	....	....	....	....	....	....	....	....	....	....	....	7	....
4	....	....	....	....	....	....	....	....	....	....	....	(a)	....	....	....
5	....	....	....	....	....	....	....	....	....	....	....	....	....	2	2
7	....	....	....	....	....	....	....	....	....	....	....	....	....	....	(a)
12	....	....	....	....	....	....	....	....	....	....	....	....	....	(a)	....
Total...	74	104	85	84	34	79	46	52	48	41	30	62	62	67	95

[a] Died.

No. 1 to 6, inclusive, emerged on October 9, 1916.
No. 7 to 11, inclusive, emerged on October 10, 1916.
No. 12 to 15, inclusive, emerged on July 27, 1917.

PARASITISM OF THE MELON FLY BY FRUIT-FLY PARASITES

Though great quantities of melon flies, *Bactrocera cucurbitae* Coquillett, were reared in the laboratory from material collected about Honolulu during the years 1914, 1915, and 1916 after the fruit-fly parasites had become established, no parasites were reared from the material until September 4, 1917. On this date, 7 normal adults of *Tetrastichus giffardianus* were reared from a single melon-fly puparium. This puparium was part of a quantity secured from cucumbers collected in Honolulu two weeks previously. Before proceeding further it should be stated that a natural parasite of the melon fly was introduced into Hawaii by Mr. D. T. Fullaway in May, 1916. It was well established by the latter

part of 1917. The rearing of these 7 individuals of Tetrastichus from the melon-fly puparium was entirely owing to the parasitism of the host, while a larva, first by this natural parasite, *Opius fletcheri* Silvestri, and later by *T. giffardianus*. Proof of this is shown by the following results.

Prior to September, 1917, many attempts were made to rear the fruit-fly parasites from melon-fly puparia. No difficulty was experienced in inducing all of the species to oviposit in the larvæ, but all parasitized individuals developed into normal flies. The opiines were easily led to oviposit into melon-fly larvæ by transferring these larvæ from various vegetables to the fruits of *Mimusops elengi*, a fruit usually heavily infested with fruit-fly larvæ. There was no need for transferring the larvæ for *T. giffardianus*, however, as it readily entered any of the hosts of the melon fly, such as cucumber, pumpkin, etc., and quickly located and oviposited into the maggots. The interesting results of these artificial attempts to rear fruit-fly parasites from the melon fly may be briefly summarized as follows:

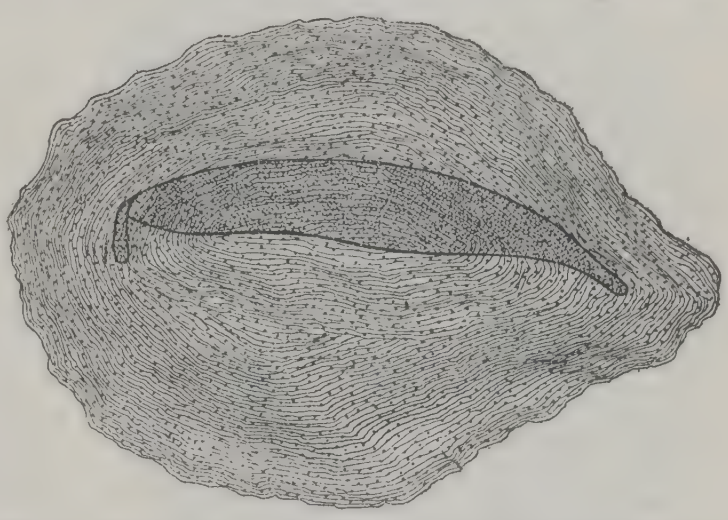

FIG. 34.—*Diachasma tryoni:* Dead encysted egg removed from melon-fly pupa. Length of capsule 0.55 mm.

During March, 1917, through the handling of melon-fly larvæ as described, a total of 154 eggs of *Diachasma tryoni* were deposited into 40 melon-fly larvæ, 17 eggs of *D. fullawayi* were deposited into 8 larvæ, 33 eggs of *Opius humilis* were placed in 8 larvæ, and 232 eggs of *Tetrastichus giffardianus* were deposited into 19 larvæ. All of these larvæ pupated normally. Some were mature when stung while others were only about two-thirds developed. From two to five days after each was stung, it was dissected, being now in the pupa stage, and the parasite eggs were counted and carefully examined. None of the opiine eggs hatched and only a small proportion of those of Tetrastichus. In every case, without exception, the opiine eggs developed only a little and then became closely encysted in a mass of transparent cellular material (fig. 34). The egg is at first only thinly surrounded with this encysting substance, but after two or three days it becomes densely inclosed, forming a homogeneous, ovoid body. The egg has then collapsed a little and has become brownish in color. It can be faintly distinguished as it lies in this body. The Tetrastichus eggs and some larvæ that managed to hatch became similarly encysted. In no case did a Tetrastichus larva live long

enough to ingest a visible quantity of food. As the Tetrastichus deposits its eggs in clusters of 8 or 10, they become "encapsulated" in such groups (fig. 35).

The presence of the eggs of these parasites in this dead, inclosed state offers no impediment to the normal development of the melon-fly larva to the adult fly. The adult emerges and carries within its body, usually in the fat masses, these eggs singly or in groups, until death. Death does not seem to be hastened by the presence of the foreign bodies within the body. On May 24 and 25, 1917, 25 melon-fly larvæ were stung by females of *Diachasma tryoni* and eggs deposited in each. The larvæ were then permitted to develop, pupate, and finally produce living adult flies. These flies were placed in large jars and kept alive for several months. On June 4, 14, and 20, July 10, August 10, and September 27, a few of these flies were killed and examined internally for evidences of the parasite eggs originally deposited in the larvæ. From 1 to 9 distinct, brownish, encysted eggs of *D. tryoni* were located in some portion of the body of each of the 25 flies on the foregoing dates.

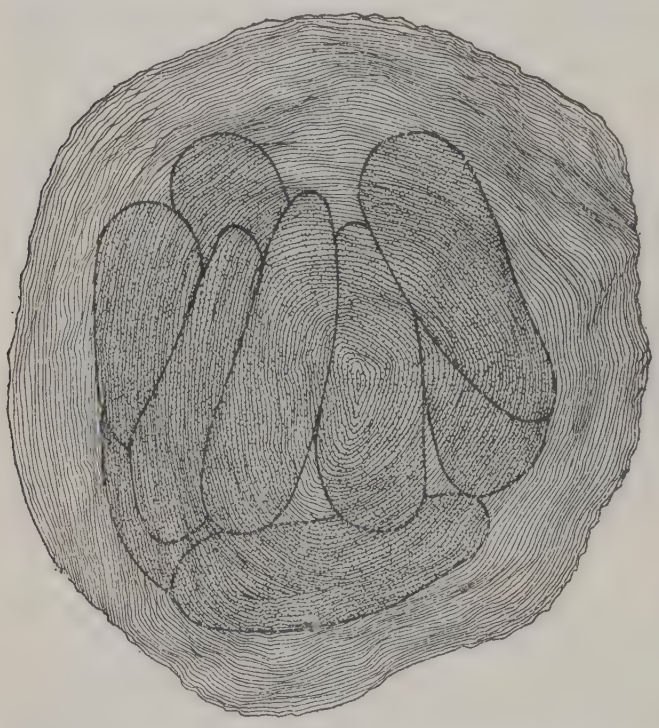

FIG. 35.—*Tetrastichus giffardianus:* Dead encysted cluster of eggs removed from melon-fly pupa. Greatest width of capsule 0.56 mm.

When, on September 4, 1917, as previously stated, 7 adults of *Tetrastichus giffardianus* were reared from a melon-fly puparium secured from the field, after such contradictory experiments had been carefully completed in March, renewed attempts were made to rear this species in the laboratory from the melon fly. The possible significance of the presence of the newly introduced melon-fly parasite, *Opius fletcheri*, was immediately recognized. As the host larva was powerless to resist the development of this parasite, it was assumed that a subsequent oviposition by *T. giffardianus* into larvæ previously stung by *O. fletcheri* might result in the rearing of adults of *T. giffardianus*. With this object in view, melon-fly larvæ were first subjected to attack by *O. fletcheri* and then by *T. giffardianus*. As the rearings and examinations from then on showed in every case where both species had parasitized the larvæ, the individuals of *T. giffardianus* developed and emerged as perfect parasites, and in every case in which only the latter oviposited

into the larva, no parasite developed and the fly matured and carried in its body the encysted eggs of *T. giffardianus*. In those cases in which both parasites oviposited, the larvæ of *O. fletcheri* always died while very young, and all of the larvæ of *T. giffardianus* survived. One case out of 201 occurred, however, wherein a female of *O. fletcheri* and 5 adults of *T. giffardianus* emerged from the same puparium. During September, October, and November, 1917, 3,092 adults of *T. giffardianus* were reared from 201 melon-fly puparia by the foregoing method of double parasitism. After all of the adults of *T. giffardianus* had emerged, microscopic examinations were made of the contents of each of the 201 empty puparia. In every case the dead larva of *O. fletcheri*, usually one to a puparium, was found. This was proof absolute that every host individual had been stung not only by *T. giffardianus* but also by *O. fletcheri*. In conjunction with this, 429 melon-fly larvæ were stung by *T. giffardianus* alone. These larvæ all matured to flies, and each of the flies when dissected was found to contain clusters of encysted dead eggs or larvæ of *T. giffardianus* in various parts of the body.

As the duration of the egg of *T. giffardianus* is shorter than is that of *O. fletcheri*, cases have been observed in which larvæ of the former were developing normally before the egg of the latter had hatched. This indicates that the immunization of the melon-fly larva against parasitic development is destroyed at the time it receives the egg of *O. fletcheri*.

This weakened resistance of the melon-fly larva to parasitic development is caused most probably by toxic substances injected into the larva by the female of *O. fletcheri* during the deposition of the egg. The reduced resistance of the larva is not caused by any mechanical injury or bacterial infection attending the insertion of the ovipositor. Larvæ have been pricked with fine cactus spines or heavily stung by *Diachasma tryoni* and then exposed to attack by *T. giffardianus*. In such cases the eggs of *T. giffardianus* invariably became encysted as usual and died. The eggs even became encysted in larvæ that had been almost killed by running a cactus spine through the body.

Unusual interest has attended the results of exposing melon-fly larvæ to the attack of *O. fletcheri*, followed by the exposure of the infested melon-fly larvæ to fruit-fly parasites other than *T. giffardianus*. In all such cases the fruit-fly parasite eggs failed to develop, becoming as tightly encysted as if the *O. fletcheri* egg had not been present. In other words, no fruit-fly parasites will develop in melon-fly larvæ under any conditions, except in the case of the eulophid *T. giffardianus*, and this species will do so only when combined with an egg or larva of the natural melon-fly parasite, *O. fletcheri*.

O. fletcheri will develop normally in fruit-fly larvæ. Laboratory experiments have proved this positively and it has been reared from fruit-fly puparia secured in the field in Hawaii on three separate occasions.

The parasitism of unnatural hosts has been investigated by others. Of Americans, the work of Mr. P. H. Timberlake is of unusual interest. He succeeded in inducing *Limnerium validum* Cresson, which is a natural parasite of the fall webworm (*Hyphantria cunea* Drury), to oviposit in caterpillars of the brown-tail moth (*Euproctis chrysorrhoea* Linnaeus), the gipsy moth (*Porthetria dispar* Linnaeus), and the rusty vaporer moth (*Notolophus antiquus* Linnaeus). In all the larvæ of *L. validum* failed to develop. Timberlake (7) states that the larvæ

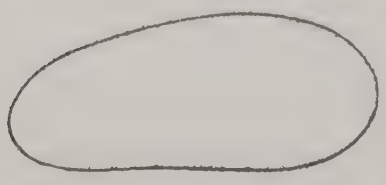

FIG. 36.—*Galesus silvestrii:* Egg, 1 day old. Length 0.36 mm.

> fail to survive the protective reactions of the host, which are visibly manifested by an accumulation of active blood cells or amœbocytes around the larvæ, the cast eggshells, and even the eggs themselves. The amœbocytes presumably attack the living eggs and larvæ, or at least ultimately efface the latter entirely.

RELATION OF THE INTRODUCED PUPAL PARASITES TO THE ESTABLISHED LARVAL PARASITES OF THE FRUIT FLY

GALESUS SILVESTRII

In addition to the larval parasites brought into Hawaii to control the Mediterranean fruit fly, a proctotrupid, *Galesus silvestrii* Kieffer, was introduced by Silvestri in May, 1913. This is a pupal parasite. Though readily breeding in fruit-fly puparia in confinement, it has never been established in the open.

FIG. 37.—*Galesus silvestrii:* Newly hatched larva. Length 1 mm.

Experiments with this species in the laboratory have shown definitely that it may act either as a primary or a secondary parasite, and that its tendencies are suspiciously more those of a secondary than of a primary parasite. Fruit-fly puparia containing developing larvæ of any of the opiine parasites above described or of *Tetrastichus giffardianus*, when exposed to attack by *G. silvestrii*, may produce adults of *G. silvestrii*. Extensive experiments with combinations of *G. silvestrii* and the larval parasites of the fruit fly have proved beyond doubt that a female of *G. silvestrii* will never place her egg loosely in a fruit-fly puparium if that puparium contains already the developing larva of some other parasite. In such cases she invariably feels about with her ovipositor until she locates the parasitic larva, into which she oviposits. This egg may hatch and ultimately a normal adult of *G. silvestrii* may emerge from the fully developed parasitic larva and bite its way out of the puparium.

Before discussing the relation of *G. silvestrii* to the other fruit-fly parasites, a brief summary of its life history will be given.

Under ordinary circumstances the Galesus female attaches herself to the fruit-fly puparium (Pl. 32, E), inserts her ovipositor, and within 3 to 15 minutes places an egg into the fly pupa. The subelliptical egg (fig. 36) hatches in 2 to 3 days. The newly hatched larva (fig. 37) is strictly an internal feeder. Its general structure is strikingly like that of the newly hatched opiine larva. The heavily chitinized head, bearing long, curved mandibles, and the soft segmented body strongly suggest a close relationship to the opiines as shown in figures 3, 4, 23, 24, and 28. No tracheæ occur in the first-instar larva. With the molt to the second instar (fig. 38), the chitinized head is lost and the body is simple, without strong characters, and very much resembles the second-instar larvæ of the opiines. A third instar occurs, resembling the second. Whether or not there is an intermediate stage between the third and the last has not been determined. With the molt to the last larval instar a strong, well-defined, open tracheal system appears, with three pairs of large stigmata. The stigmata are borne on the first three segments back of the head. Heavily chitinized, sharply pointed mandibles are present (fig. 39). No waste material is voided by the larva. The midintestine is filled with this waste and is closed caudally. Just before pupation, in the prepupal stage, the entire waste accumulation is discharged. Into this meconium the exuvium of the mature larva is shed upon pupation. The pupa then lies with the caudal tip embedded in the voided meconial mass and the crumpled larval exuvium. The entire egg, larval, and pupal period averages, in Honolulu, from 25 to 32 days, depending upon the temperature. The adult emerges usually by pushing off the dorsocephalic cap of the puparium (fig. 40). Mating and oviposition occur immediately upon emergence. The female flies

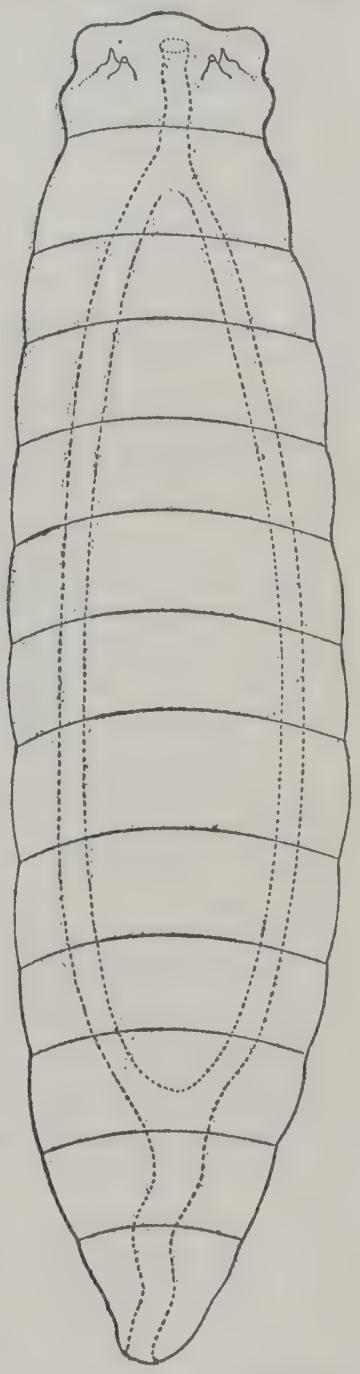

Fig. 38.—*Galesus silvestrii:* Larva of second instar. Length 1.5 mm.

Fig. 39.—*Galesus silvestrii:* Mandible of mature larva. Length 0.085 mm.

very little. It remains about the ground, entering crevices and crawling under various objects, apparently in search of fly puparia. In Hawaii it has been reared from the puparia of several muscid species, but it has never been reared from any material collected in the field. The female may reproduce parthenogenetically, the resulting progeny being males.

In the laboratory the female does not discriminate between parasitized and unparasitized fly puparia. She will insert her ovipositor into any puparium. If it contains only a developing fruit-fly pupa, the egg is deposited into the pupa. If the puparium contains a parasitic larva, which may be even one of the small Tetrastichus larvæ, her ovipositor will search out this larva and an egg will be deposited into it. A Galesus adult ultimately may mature within this larva. It will, of course, be dwarfed. These small adults are fertile. Such a habit indicates a strong hyperparasitic inclination.

During August, 1917, a total of 195 fruit-fly larvæ were parasitized in the laboratory by *Diachasma tryoni* or *Opius humilis*. These larvæ were then permitted to develop and to form into perfect puparia. The puparia were then immediately placed in cages containing females of *G. silvestrii* and left for 2 days. From 5 to 7 days later each puparium was opened and the contents examined. In every case the puparium contained a dead opiine larva or occasionally a pupa, in each of which was a rapidly developing, strong larva of *G. silvestrii*. This, then, was hyperparasitism pure and simple. To prove that these larvæ of *G. silvestrii* ultimately would develop to normal adults, a fresh lot of heavily parasitized fruit-fly larvæ was collected in the field. They were variously parasitized by *D. tryoni*, *D. fullawayi*, *O. humilis*, and *Tetrastichus giffardianus*, and some were unparasitized. The puparia thus formed from the larvæ were placed with 75 adults of *G. silvestrii* on September 26 and left for 24 hours. The puparia, 277 in number, were then removed and each placed separately in a small vial to await emergence. From this material 94 adults of *G. silvestrii* emerged, with both sexes well represented. Each of the 94 puparia producing the adults of *G. silvestrii* was then carefully dissected, opened, and examined. In 68 of the puparia the empty shell of a mature *D. tryoni* larva was found, and in each shell the cast mandibles of the larva of *G. silvestrii* as well as the characteristic prepupal meconial discharge of *G. silvestrii* was disclosed. In 3 puparia a similar larval shell of *O. humilis* was found, each with similar contents. In 1 puparium the shell remains of a mature *D. fullawayi* larva was found, and in it also were the larval mandibles and prepupal meconial discharge of *G. silvestrii*. In the remaining 22 puparia which had produced adults of *G. silvestrii* remains of the

Fig. 40.—*Galesus silvestrii:* Fruit-fly puparium showing characteristic emergence hole made by adult parasite. Greatly enlarged.

fruit-fly pupa alone occurred, within which were the larval remains of *G. silvestrii*. The remaining 183, puparia not having been parasitized by *G. silvestrii*, produced 52 adults of *D. tryoni*, 14 of *O. humilis*, 1 of *D. fullawayi*, 12 of *T. giffardianus*, 3 fruit flies, and the balance dried up and produced nothing. Thus, in the 94 cases above cited, 72 occurred in which a normal adult of *G. silvestrii* emerged as a hyperparasite on an opiine parasite of the fruit fly. In 22 other cases it emerged as a normal primary parasite of the fruit fly.

As above mentioned, females of *G. silvestrii* will always oviposit into larvæ of *Tetrastichus giffardianus* as they lie developing in the fruit-fly puparium. *T. giffardianus* is a small parasite, being hardly one-half as large as *G. silvestrii*. Its larvæ are thus very small to receive the comparatively large egg of *G. silvestrii*. Larvæ of *T. giffardianus* about 0.7 mm. long are oviposited into by *G. silvestrii*, and the egg, which is about 0.35 mm. long, hatches and the larva of *G. silvestrii* develops, pupates, and may ultimately produce a dwarfed adult about 1.5 mm. long. The immature larva of *T. giffardianus* receiving the egg of *G. silvestrii* is able to develop to maturity. It is killed, however, before it is able to pupate. Females of *G. silvestrii* have actually oviposited into larvæ of *T. giffardianus* only 2 days old. In all of the experiments conducted to prove the hyperparasitic action of *G. silvestrii* over *T. giffardianus*, the eggs of the former were deposited into larvæ of the latter not more than 4 days old, the majority being only 2 and 3 days old.

On August 28 and 29, 1917, a total of 209 mature fruit-fly larvæ were exposed to attack by *T. giffardianus*. All of these larvæ formed into puparia during the next two days. On August 31 these 209 puparia were placed in a jar containing about 100 females of *G. silvestrii* and left until September 2. On September 5, 6, and 7, 59 of the puparia were opened, examined, and the contents carefully recorded. Each puparium contained from 3 to 19 well-developed larvæ of *T. giffardianus*, and in every case from 1 to 6 of these larvæ were dead and contained an active larva of *G. silvestrii* in the first, second, or third instar. The remaining 150 puparia were saved for adult emergences and ultimately produced fruit flies, adults of both species, and in some cases both species from the same puparium.

Thus we have positive proof that this proctotrupid may be strongly hyperparasitic upon any of the established parasites of the fruit fly that are now contributing toward its control in Hawaii, and that it may act with equal freedom as a primary parasite of the fruit fly.

PACHYCREPOIDEUS DUBIUS

A pteromalid, *Pachycrepoideus dubius* Ashmead, introduced from the Philippines by Mr. D. T. Fullaway in 1914 as a dung-fly parasite, has

been reared occasionally from fruit-fly puparia. It is not an important fruit-fly parasite, but the interesting relations that may exist between this and the other fruit-fly parasites has led to a laboratory study of this species, with results worth recording.

The female oviposits into the puparium, the egg being placed on the surface of the developing pupa within, and its larva develops on the pupa as an external feeder. Thus from the first it bears a well-developed tracheal system. The fly pupa is killed shortly after the parasitic larva hatches and the larva continues to feed upon the decomposing pupa. It feeds much in the sense of a scavenger. It does not consume all of the food in the puparium. The meconium is voided just before pupation and the pupa lies partly buried in the decayed remains of the fly pupa and its own meconium. The adult parasite emerges by cutting a small, circular hole in the puparium (fig. 41) and is immediately ready for mating and oviposition. Another minute meconium is discharged immediately upon emergence. The female may reproduce parthenogenetically with the resulting progeny all males. The entire period from deposition of egg to emergence of adult lasts only from 15 to 16 days.

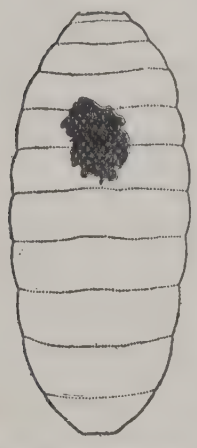

FIG. 41.—*Pachycrepoideus dubius:* Fruit-fly puparium showing characteristic emergence hole made by adult parasite. Greatly enlarged.

As the larva of this parasite is an external feeder within the puparium from the first, and the larvæ of the other parasites are all internal feeders, the relationship between them is interesting. In a certain sense this parasite has been proved a primary, a secondary, or a tertiary parasite. As a primary it develops on the fruit-fly pupa, as a secondary it develops on larvæ or pupæ of *Tetrastichus giffardianus*, *Opius humilis*, *Diachasma tryoni*, *D. fullawayi*, or *Galesus silvestrii* as they occur as parasites in the fruit-fly puparium, and as a tertiary parasite it will of course develop on a larva of *G. silvestrii*, which in turn has been feeding in the larvæ of the above-mentioned opiines or *T. giffardianus*. In the laboratory, in large jars, this parasite indiscriminately oviposits into fruit-fly puparia to bring about all of the above conditions of parasitism and may be expected to act similarly in the field, when able to reach fruit-fly puparia. Fortunately it does not show any decided capacity for penetrating soil in search of fly puparia and is probably more attracted to situations harboring the dung-feeding species. The data demonstrating the various capabilities of this species as a primary, secondary, or tertiary parasite may be summarized as follows:

During November 24 and 25, 1917, a total of 341 fruit-fly puparia were secured from kamani fruits collected about Honolulu. These puparia were variously parasitized by *O. humilis*, *D. tryoni*, *D. fullawayi*, and *T. giffardianus*. On November 29 they were placed in a jar con-

taining about 100 females of *P. dubius* and left until December 2. They were then removed and on December 7 and 8 dissected and carefully examined. Thirty-one contained a dead larva or pupa of *D. tryoni* on which was feeding a mature larva of *P. dubius;* 73 contained a dead larva or pupa of *O. humilis* on which fed a larva of *P. dubius;* 2 contained a dead larva of *D. fullawayi* on which was a larva of *P. dubius;* 22 contained from 3 to 31 larvæ of *T. giffardianus*, some of which were in each case dead and on which fed a larva of *P. dubius;* 30 contained decomposed fruit-fly pupæ parasitized by *P. dubius* alone. Of the remaining 183 puparia, 46 were unparasitized, 88 were parasitized by only *O. humilis*, 31 by only *D. tryoni*, and 18 by only *T. giffardianus*.

Again, on October 23, 50 fruit-fly puparia containing developing larvæ of *O. humilis* and *D. tryoni* were exposed to attack by *G. silvestrii* for one day. On October 30 they were placed in a jar containing females of *P. dubius* and left for two days. They were then opened and examined on November 10 with the following interesting results: Thirty-five contained dead larvæ of *G. silvestrii* lying in dead larvæ of *O. humilis* or *D. tryoni*, on the whole of which was feeding in each case a larva of *P. dubius*.

The relation of the *P. dubius* to *T. giffardianus* is also of interest. Very frequently a puparium containing 15 or 20 larvæ or pupæ of *T. giffardianus* and exposed in the laboratory to attack by *P. dubius* will yield a normal adult of *P. dubius* and several normal adults of *T. giffardianus*. On November 23 and 24, 83 fruit-fly larvæ were parasitized by *T. giffardianus* in the laboratory. The 83 puparia forming from these were then placed with adults of *P. dubius* and left for three days. On December 8 the puparia were opened and examined. In 23 cases examined, from 4 to 20 dead larvæ of *T. giffardianus* were found, and on these in each case was feeding a mature larva of *P. dubius;* in 30 puparia from 1 to 10 living pupæ of *T. giffardianus* occurred together with from 3 to 20 dead larvæ of that species upon which were feeding larvæ of *P. dubius*, one in each instance; in the remaining 33 puparia from 7 to 19 individuals of *T. giffardianus* were developing in the absence of individuals of *P. dubius*.

In conclusion, it should be borne in mind that *G. silvestrii* is not known to be established in Hawaii as yet, and that *P. dubius* probably only parasitizes a fraction of 1 per cent of the fruit-fly puparia in the field, but that the relations between these and the other fruit-fly parasites, as detailed in the foregoing pages, can be expected as a natural sequence if they ultimately adapt themselves to Hawaiian conditions and become thoroughly established.

PHEIDOLE MEGACEPHALA AS A PREDACIOUS ENEMY OF THE FRUIT FLY

Although several species of ants are common in the Hawaiian Islands, the cosmopolitan ant *Pheidole megacephala* Fabricius greatly outnumbers all others and is enormously abundant throughout the islands at low elevations. It is quite probable that it checks and greatly limits the increase of several of these species. It is quickly drawn to any fresh or decaying animal matter and the soft body of the fruit-fly larva falls an easy prey to it. It has been generally assumed that this ant destroys large numbers of fruit-fly larvæ throughout the year. Very little definite data are on record, however, covering investigations on this relationship. Though the following records are not extensive, they all point in one direction and are significant.

Four hundred and eighty-eight kamani fruits collected from a certain tree in Honolulu on December 3, 1916, were spread out under the tree on a large piece of canvas covered with 1 inch of sand and left for 10 days. By this time practically all of the contained fruit-fly larvæ had developed and pupated in the sand beneath the fruits or had been carried away by ants. The sand was then sifted and the fruit-fly puparia were counted. A total of 606 fruit-fly puparia were obtained. This was an average of 1.2 larvæ per fruit. During November and December 969 more fruits were gathered from the same tree and taken to an ant-proof insectary to be held in boxes of sand for 10 days. From these 4,895 fruit-fly puparia were obtained, or an average of 5 larvæ per fruit. Here four times as many larvæ per fruit developed in the lot where the ants were excluded. From 1,301 fruits of the same kind collected from another tree in Honolulu in November, 1917, and similarly placed and held for 10 days on canvas under the tree, 3,742 fruit-fly puparia were ultimately obtained. From the same tree and collected at approximately the same date, 909 fruits were taken and brought to the ant-proof insectary. From these 10,119 fly puparia were secured. Here again almost four times as many larvæ per fruit were obtained from the fruits held away from the ants. On January 25, 1918, 664 loquats were collected from several trees in a certain yard in Honolulu and divided in half, one half going out on the canvas and the other part to the insectary. From the lot on the canvas, after 10 days' exposure, 156 fruit-fly puparia were gathered, and from those in the insectary a total of 840. In this case the ant-proofed lot produced over five times as many puparia. On January 29, February 2, and February 6, 1918, an aggregate of 3,100 fruits of the black myrobalan was collected from one tree. This total was divided in half as soon as each lot had been collected, the two portions being handled as in the above cases. From the fruit held in the insectary 6,668 fly puparia were obtained, while only 4,385 puparia were ultimately secured from the half placed out on

the canvas. Here the lot held away from the ants was 1½ times as great as that exposed to the ants.

The foregoing should represent a very conservative estimate of the destruction of fruit-fly larvæ by this ant. It shows consistently that from one-third to four-fifths of the larvæ developing in all fruits in the field very probably never mature to adult flies.

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PLATE 32

Oviposition of fruit-fly parasites:

A.—*Diachasma tryoni* ovipositing into fruit-fly larva in fruit of *Mimusops elengi*.
B, C, D.—*Tetrastichus giffardianus* ovipositing into fruit-fly larva.
E.—*Galesus silvestrii* ovipositing into fruit-fly puparium.

A
B
C
D
E

OBSERVATIONS AND EXPERIMENTS ON INTESTINAL TRICHINÆ

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SCOPE OF WORK

Although *Trichinella spiralis* has been studied by many investigators, the literature on the subject is almost exclusively devoted to the morphology and life history of the parasites and their relation to the disease which they produce in man and in other mammals. Several investigators have recorded some casual observations on the physiology of these parasites, particularly with reference to the resistance of the encysted larvæ to unfavorable conditions. No attempt has heretofore been made, however, to present a systematic account of the behavior of these organisms under the influence of various physical and chemical stimuli at different stages of their life history. Recently Ransom (*6*)[1] in the course of a report of an investigation on the effects of refrigeration on the encysted larvæ of *Trichinella spiralis* has presented some interesting data relative to their behavior when freed from their cysts by artificial digestion. Ransom and the present writer have continued these investigations and have obtained a considerable amount of data[2] on the resistance of the larvæ to high temperatures and to various physical and chemical agents.

Inasmuch as little has been known heretofore concerning the physiological processes of the parasites concomitant with their growth in the small intestine, it appeared to the writer that observations and experiments on the intestinal forms, with special reference to their behavior under various conditions, might yield some useful information. The present paper embodies the results of this work and also includes observations on the molting of the larvæ *in vitro*, a phenomenon which, so far as the writer is aware, has not been hitherto recorded.

METHODS OF STUDY

In nearly all the observations and experiments recorded in this paper the parasites were obtained from albino and hooded rats which were artificially infected by feeding ground-up trichinous pork. It was found that after being kept on a vegetable diet for several days, rats devour meat very readily even though it is badly decayed. In some respects

[1] Reference is made by number (italic) to "Literature cited," p. 482.

[2] These results have not yet been published.

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rabbits and guinea-pigs are less favorable hosts from the experimenter's point of view. They will not swallow meat unless it is fed forcibly. Even under these conditions the attempted infection is not always successful in the case of rabbits, and occasionally yields negative results in guinea-pigs. The former were observed to regurgitate meat several minutes after being fed. However, aside from these considerations the size of the intestine and the character of its contents make it rather difficult to isolate the parasites from the intestine of rabbits and guinea-pigs. The intestine of the rat, being considerably smaller, presents the parasites in greater concentration. In the writer's experience the contents of the intestine of a rat even a few hours after a meal do not seriously interfere with the detection and isolation of the parasites. However, when it is desired to keep the host animals alive for several weeks, rats are not to be relied upon unless they are only lightly infested with the trichinæ and are kept in a warm place and protected from drafts. A heavy invasion of trichinæ sets up an acute intestinal inflammation in rats and causes their death as early as the third day after artificial infection. A sudden change in temperature or a draft is very likely to bring them down with pneumonia. If it becomes necessary, therefore, to keep the hosts alive for a month or longer, rabbits or guinea-pigs should be used in preference to rats. Instead of attempting to feed the meat forcibly to these animals, the writer found it more desirable to use the following procedure: The meat is thoroughly chopped up and then digested in an artificial gastric juice for about 18 hours at a temperature of 38° to 40° C. The formula for artificial gastric juice recommended by Ransom (*6*) has proved entirely satisfactory except that it was found that by reducing the amount of sodium chlorid to 2 grams per 1,000 cubic centimeters of fluid, a more thorough digestion of the meat was obtained.[1] After digestion of the meat the fluid is slowly decanted and replaced by a 0.7 per cent solution of sodium chlorid. The parasites are allowed to settle to the bottom, and the supernatant fluid is again decanted. This may be repeated until the parasites have been thoroughly washed. They may then be transferred to a smaller vessel and taken up in a fine pipette with some of the salt solution and forced down the esophagus of rabbits or guinea-pigs. This procedure has invariably resulted in a successful infection.

Intestinal trichinæ were obtained from their hosts as follows: After the animal had been killed by chloroform, the intestine was removed to a large petri dish containing a 0.7 per cent solution of sodium chlorid. The intestine was then slit open from end to end and the mucosa carefully scraped with the dull edge of a scalpel in order to remove the parasites from their places of attachment. They were then picked out with the aid of a dissecting microscope by means of a fine pipette, the opening

[1] The modified formula is as follows: Water, 1,000 cc.; hydrochloric acid (sp. gr. 1.19), 10 cc.; scale pepsin (U. S. P.), 2.5 gm.; sodium chlorid, 2 gm.

of which was too small to admit the coarser intestinal débris. The organisms were transferred to a 0.7 per cent solution of sodium chlorid in a watch glass which could be mounted on the stage of the microscope in order to make observations. To make out details slide preparations were made. When it was desired to keep the parasites in the laboratory overnight they were transferred by means of the pipette to a second watch glass in order to get rid of as much intestinal débris as possible. If this precaution is not taken putrefaction may set in, which, besides causing an offensive odor, forms a film on the surface and interferes with the observations.

Unless otherwise stated the parasites were kept in a 0.7 per cent solution of sodium chlorid which will be referred to as a physiological salt solution. In the description of the experiments and observations reference is made to the time which elapsed between the feeding of the trichinous meat to the host and the isolation of the parasites from the intestine. Inasmuch as albino rats do not as a rule eat the meat as soon as it is offered to them allowance must be made for the intervening period, which may vary from a few minutes to several hours.

MIGRATION OF THE LARVÆ FROM THE STOMACH TO THE INTESTINE

When trichinous meat is fed to a suitable host the cysts are digested in the stomach, the larvæ becoming free in the lumen. The following observations point to the conclusion that the decapsuled larvæ do not linger in the stomach but make their way into the small intestine very promptly after their liberation from the cysts. Furthermore, to judge from their behavior *in vitro*, it appears highly probable that their own muscular movements aid them in this change of location.

When the stomach still contains trichinous meat in various stages of digestion few decapsuled larvæ are found in it, although the small intestine may contain many newly arrived parasites. The latter may still be tightly coiled and, therefore, probably unattached to the mucosa. Within four hours after feeding trichinous meat, a number of tightly coiled larvæ were found in the intestine, but none were seen in the stomach. Eighteen hours after feeding, the small intestine was found to contain numerous parasites, some attached to the mucosa and active, others still unattached and coiled, the latter lying free in the lumen. The stomach contents of such hosts were usually found to be free from parasites. Feeding trichinous meat in which the parasites are no longer alive or so reduced in vitality as a result of refrigeration or other means of attenuation that they fail to survive the action of the gastric juice, does not result in a rapid transfer of the larvæ from the stomach into the intestine. Within about 18 hours after feeding such meat numerous larvæ were found in the stomach. The parasites were uncoiled and inactive and somewhat paler than larvæ known to be alive. In the small intestine the parasites were found in various stages of digestion.

Ransom (*6*) has shown that after trichinous meat has been digested at a temperature of 37° to 40° C. in an acidified solution of scale pepsin the parasites are liberated from their cysts. When taken out of the incubator the larvæ in the artificial gastric juice are found to be very active. Their activities are due to vigorous muscular movements, which propel the parasites and are thus locomotory in nature. Assuming that after liberation from their cysts in the stomach of a rat or other host the larvæ exhibit the same activity, it is probable that their own movements hasten and guide their transfer to the intestine. Chemotactic influences may play an important part in the migration of the larvæ from the stomach to the intestine.

The nonliving larvæ apparently remain in the lumen of the stomach until they are forced into the intestines with the chyle.

EFFECTS OF GASTRIC DIGESTION ON THE LARVÆ

Ransom (*6*) found that after artificial digestion the decapsuled larvæ may be kept active for over three weeks in a physiological salt solution. Ransom and the writer kept decapsuled larvæ alive in Ringer's fluid at a temperature of about 10° C. for seven weeks, at the end of which period they showed activity on a warm stage.[1] At body temperature the life of the decapsuled larvæ kept *in vitro* is brief according to the writer's observations, seldom exceeding 48 hours. But whether the larvæ survive several weeks or several hours, they neither increase in size nor exhibit any other morphological changes which can be attributed to growth. After artificial digestion they can maintain their vitality outside of the host for varying periods, depending on the temperature and osmotic pressure of the medium in which they are kept, but they do not resume their processes of growth and morphogenesis, which came to a halt coincident with encystment.

The question arises whether natural gastric digestion not only releases the parasites from their cysts, as artificial digestion does, but also in some way stimulates them to growth and development. That is, may not the impulse which leads to growth originate in the stomach of the host, the intestine merely supplying a suitable environment for continuation of the process begun in the stomach? Within four hours after feeding trichinous meat the writer isolated several larvæ from the small intestine. They were tightly coiled and apparently quite unaffected by their passage through the stomach and brief sojourn in the intestine. After being transferred to a physiological salt solution they were placed in a refrigerator at 10° C. They remained tightly coiled in the same way as larvæ obtained by artificial digestion.

Coiled trichinæ obtained from the intestine 15 hours, 18 hours, and 24 hours after feeding trichinous meat were similarly isolated and kept

[1] The results of these experiments have not yet been published.

alive in a physiological salt solution for a number of days without their undergoing any growth changes. These larvæ must have been in the small intestine for at least several hours, yet their larval condition remained unaltered and they were capable of living for several days in a salt solution. In view of these facts and in the light of what will presently be described with reference to the behavior of the intestinal parasites which have begun further development, it is apparent that the effects of natural gastric digestion on the larvæ is very much like the effects of artificial digestion; that is, the parasites are liberated from their cysts, but are not stimulated to further development.

CHANGES IN THE BEHAVIOR OF THE DEVELOPING LARVÆ

As has already been stated, larvæ taken from an artificial digestive medium after a period of incubation of 18 hours are quite active. Even after being washed in a physiological salt solution several times, the parasites still exhibit movements at room temperature. Gradually they become sluggish, the posterior end of the worm ceasing its contractile movements entirely, while the anterior end may still exhibit feeble activity. The worms finally coil up tightly and lapse into a quiescent condition, which characterizes these parasites in their normal locations in the voluntary muscles of the host.

In comparison with the quiescent state of the intact larvæ at room temperature the sexually mature and the maturing parasites taken from the small intestine and kept under observation in a physiological salt solution present a marked contrast. The intestinal forms continue their movements with almost unabated vigor until they succumb. Their activities are not as feverish as those of the intact larvæ which have been artificially stimulated, but are more persistent, more regular, and independent to a considerable extent of any external stimulation. Under none of the conditions to which they were subjected have the intestinal trichinæ been observed to coil up. Not even the depressing temperature of a refrigerator (10° C.) caused them to manifest the least traces of becoming coiled. The cold rendered them temporarily rigid, but they remained either completely elongated or exhibited a sinuous outline. It was quite evident that the low temperature paralyzed their movements, leaving each individual in the posture which it had assumed prior to coming under the influence of the cold.

It appears, therefore, that in addition to certain morphological differences the intestinal forms may be readily differentiated from the intact larvæ by their behavior *in vitro*. This is not the only physiological criterion, however, which can be employed to distinguish the maturing forms from the encysted larvæ. The latter have been known for a long time to have marked powers of resisting various toxic agents, in which respect they also stand out in sharp contrast with the developing forms,

as will be shown elsewhere in this paper. The last criterion can therefore be employed as a check on the first to distinguish the early developing intestinal larvæ from the intact forms, particularly when the morphological state following growth in the intestine is not yet sufficiently advanced to warrant a diagnosis on the basis of structure.

In order to determine whether the change in the behavior of the larvæ becomes established coincident with the resumption of growth in the small intestine, the organisms were taken from their hosts within 18 to 24 hours after feeding trichinous meat. Upon examination some of the parasites were seen to be in process of molting, and their subsequent behavior at room temperature showed that individuals which have reached the molting stage have abandoned the larval habit of becoming coiled. Of the nonmolting larvæ, some continued their active movements, whereas others became sluggish and finally coiled up. A number of active forms molted while they were kept under observation, and many of the molting as well as the nonmolting forms succumbed within a few hours. The quiescent forms which became coiled neither resumed their activities spontaneously nor perished at room temperature. Some of these forms were stored in a refrigerator where they maintained their vitality for many days. In fact, they behaved in the same way as larvæ obtained by artificial digestion of trichinous meat.

In view of the fact that certain larvæ obtained from the host within 18 to 24 hours after feeding trichinous meat may already have molted at least once in the intestine, it is safe to assume that the active larvæ which did not molt while they were kept under observation had already cast off their cuticles in the intestine of the host. At least their behavior *in vitro* showed that they are to be classed with the actively molting forms.

These observations show that larvæ which are in the act of molting, as well as larvæ which have molted prior to their isolation from the intestine, have lost the power of assuming the tightly coiled posture which characterizes the intact larvæ. They continue their movements until they succumb, and exhibit other traits characteristic of larvæ which are definitely known to have molted. Thus they may undergo rapid disintegration when kept in a physiological salt solution at room temperature and exhibit an intolerance for certain toxic substances, which will be discussed elsewhere in this paper.

MOLTING OUTSIDE OF THE HOST

Larvæ taken from the intestine within 18 hours after feeding trichinous meat do not as a rule show any evidence of having molted. If the individuals that continue their movements one or two hours after isolation from the small intestine are carefully observed, the molting process may be studied from its initial stage to its completion. The first evidence is

a retraction of the larva from its cuticle, leaving the latter empty at one end. This is usually observed to occur first at the posterior end. The empty portion continues to increase in size, and gradually the anterior end of the worm begins to contract in the same manner as the posterior end. The worm now assumes the appearance of being incased in a cuticle which is a little too long for it. The head end usually breaks through the membrane and continues lively movements until the entire worm pulls out, leaving the cast-off skin behind.

It is a significant fact that certain larvæ isolated from the small intestine as late as 24 hours after feeding the host trichinous meat resume their coiled-up larval posture and do not undergo any further changes when kept in physiological salt solution, whereas others under the same conditions continue their lively movements and undergo a molt. The active larvæ have apparently been stimulated by some factors present in the small intestine. Their greater activity at room temperature as compared with that of intact larvæ is evidence that their metabolism has been accelerated, and that this change in the rate of metabolism is nonreversible is evident from the fact that the parasites no longer react in the former manner to a lowering of the temperature, but continue active. Their subsequent molting would indicate that the influences under which the parasites resume their processes of growth and morphogenesis are so strong that having once been initiated the reaction proceeds even though the environment under which it normally occurs is replaced by an inert solution of sodium chlorid. The decapsuled larvæ prior to their stimulation in the small intestine are in a condition of physiological stability. They may, of course, be artificially stimulated to abnormal activities by various physical and chemical agents which if of sufficient intensity and duration soon result in their total destruction. But when the larvæ are mildly stimulated, the reactions usually subside shortly after the exciting cause is removed, and the larvæ again lapse into a quiescent state. Artificial physical and chemical stimuli, short of those that destroy the life of the parasites therefore produce reversible reactions. Under the influence of conditions to which the larvæ are subjected after reaching the small intestine of the host animal, a series of reactions is initiated which produce morphological changes in the parasites, leading toward their sexual maturity. These reactions are nonreversible, as they continue for a relatively long period of time in the absence of conditions that brought them about.

The molting process in the larvæ proceeds rather slowly at room temperature. At a lower temperature it may be suppressed, whereas at body temperature it may be greatly accelerated, as the following experiment will show:

Larvæ were obtained from a rat within 24 hours after feeding trichinous meat. A few parasites were placed in each of three watch glasses containing a physiological salt solution, and kept, respectively, in an incu-

bator at 40° C., in a refrigerator at 10° C., and at room temperature. At the end of about three hours each watch glass was examined and the following results noted: The larvæ from the refrigerator were rigid though not tightly coiled. After standing at room temperature for several minutes they became active, but no evidence of molting could be found. A careful examination of the contents of the watch glass failed to show any cast-off cuticles. The larvæ which had been left at room temperature were in the act of molting, but with the possible exception of two or three, the process had not been completed. The larvæ from the incubator were feverishly active and nearly all of them had completely cast off their cuticles, which were found at the bottom of the dish. Some were in the act of molting again.

Thus it is evident that temperature is a prime factor in the growth of the intestinal trichinæ. It is rather significant that a temperature of 10° C. inhibited the growth process without exerting a deleterious effect on the larvæ. In fact, after removal to room temperature one of the larvæ which had been kept in the refrigerator was seen to commence to molt, but as the observation was not continued it is not known whether the process was completed. The life processes of *Trichinella spiralis* under experimental conditions are modifiable to a considerable extent by certain environmental factors. Within certain limits they may be retarded by low temperatures and accelerated by high temperatures after the parasites are taken out of their host.

In the above experiment it was noted that certain larvæ behaved quite differently from the others. At room temperature as well as in the incubator they remained tightly coiled. The parasites in the dish taken from the incubator were all active at first, but at the end of about 30 minutes a number of larvæ became sluggish and finally coiled up. The assumption was made that the coiled larvæ were still intact so far as further growth was concerned and that they had apparently remained uninfluenced by their stay in the intestine. It was obvious that the incubator temperature did not stimulate them to development, but in order to settle this point more definitely the following experiment was performed.

A rat was killed within 20 hours after feeding trichinous meat. The larvæ after being isolated from the intestine were allowed to remain at room temperature for about an hour and only those which became tightly coiled were isolated and placed in another watch glass. The latter was kept under observation for about 30 minutes, and those larvæ which were not definitely coiled were taken out by means of a pipette. The dish was then covered and placed in an incubator at 40° C. for three hours. When the parasites were removed to room temperature they were quite active. Gradually their activities ceased, and they began to coil up. No cast-off cuticles were found in the dish and no parasites were seen to be molting.

It would therefore seem that it is not the high temperature which is responsible for the rapid molting of the larvæ, but that it merely hastens the process commenced in the small intestine. Larvæ which by their behavior appear to have escaped the intestinal influences do not molt even under a favorable temperature.

SURVIVAL OF INTESTINAL TRICHINÆ OUTSIDE OF THE HOST

One-day-old intestinal trichinæ are sensitive to the sudden change of environment and die within a few hours after their transfer from the intestine to physiological salt solution, either by a gradual process of disintegration in which case there is a dissolution of the worm progressing from one end to the other, or else they become rigid and begin to undergo granular degeneration without dissolution. It was found that such parasites do not even survive for 24 hours in a refrigerator at a temperature of about 10° C. Two-day-old intestinal trichinæ are not quite so sensitive and may be kept alive for 24 hours at 10° C. but not much longer. In 3-day-old intestinal parasites the resistance is greater. They have been kept alive in a refrigerator for two days, though at room temperature they succumbed earlier. Four-day-old and older intestinal trichinæ may be kept alive without difficulty for several days at a low temperature. Thus after development has actually begun in the intestine the tolerance to unfavorable conditions increases in proportion to the age of the parasite. The larvæ before they have been affected by their presence in the intestine are highly resistant to unfavorable conditions. On the other hand the youngest intestinal forms, shortly after the resumption of growth and morphogenesis, can not withstand abrupt changes which interfere with their developmental processes. The sexually mature forms again pass into a condition of relative stability which is accompanied by an increase in the power of resistance to unfavorable surroundings.

These observations are in harmony with the observations on the survival of the intestinal trichinæ after the death of their host. The writer found that within about 12 hours after the death of the host 1- or 2-day-old intestinal trichinæ usually perish, though occasionally a few may remain alive. They lose their hold on the mucosa and lie free in the lumen of the intestine where they undergo granular degeneration. Older intestinal trichinæ survive for a longer period, since they have been found still alive after the host had already begun to undergo decided putrefactive changes.

SPONTANEOUS DISINTEGRATION OF INTESTINAL LARVÆ IN VITRO

In the course of the observations on the parasites removed from the intestine after artificial infection it was noted that when the larvæ are kept in salt solution at room temperature some of them begin to disintegrate spontaneously. In fact the writer observed on several occasions

that watch glasses which but two hours earlier contained numerous larvæ had become almost free from the worms. This phenomenon was so striking that it was studied in detail as to the method of occurrence. The worms utilized in these observations were obtained from the host 24 hours after artificial infection. For the first 2 or 3 hours after the larvæ were isolated and kept at room temperature, the phenomenon was but seldom observed. Later, epidemics of disintegrations were noted, and worms at various stages of degeneration were readily found. The first sign of the process is a granulation of the worm at one end, more often at the anterior end. This is followed by a disappearance of the granules, the worm becoming gradually smaller. Occasionally the writer noted parasites in which the two ends had degenerated and the middle part was still intact. The wave of disintegration spreads slowly, and involves not only the internal organs, but the cuticle as well. In this respect, as will be shown later, the process differs from disintegration induced by potassium cyanid.

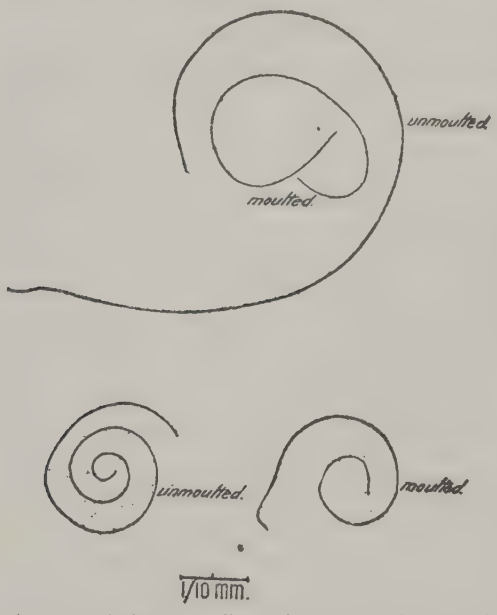

FIG. 1.—Skeleton outlines of two dwarfed trichinæ after the first molt outside of the host and of two unmolted larvæ from the same host about 18 hours after artificial infection.

The writer was at first inclined to believe that the absence of the intestinal contents of the host was perhaps responsible for the degenerative changes of the worms. However, even when the worms were kept in salt solution to which the intestinal contents of the host were added the process still occurred.

Several interesting speculations suggest themselves in this connection. The degeneration may perhaps be associated with a failure to undergo successful molting. It is not at all improbable that worms which have been stimulated by certain factors of the intestinal environment should, after sudden removal from that environment, undergo degenerative changes. There is a further possibility that the lack of proper food may check the impulse to growth and development, and lead to the process of degeneration. Finally, it is possible that concomitant with the growth of these parasites they liberate toxic substances which in an artificial environment exert a deleterious effect upon the parasites themselves. The last hypothesis can be subjected to experimental verification and the writer hopes to study this phase of the subject in connection with studies which are now in progress on the possible presence of toxic substances in the sera of hosts harboring trichinæ. It may be mentioned that Tallqvist (7) found in *Bothriocephalus latus* a proteolytic ferment which was capable of destroying the proglottids of that tapeworm.

DWARFS PRODUCED IN CULTURE DISHES

In the course of the observations on the molting of the larvæ in culture dishes it was found that sometimes after the first molt the parasite is only a little more than one-half the size of the unmolted larva (fig. 1, 3). These dwarfs maintain their vitality for some time after the molt, although they appear to be exceedingly sluggish. Their tissues, too, are not as transparent as the tissues of normal larvæ after the first molt. In fact the worm as a whole is darker in color and presents a very compact appearance. It is possible that the decrease in size is a result of food deprivation involved in the removal of the parasites from their normal environment to a culture dish. It is rather remarkable that under such conditions a second molt should be initiated almost immediately after the casting off of the first cuticle. The outline of the worm shown in figure 3 is of a dwarf larva of *Trichinella spiralis* in the course of the second molt. The parasite was obtained 18 hours after artificial infection, and the observation was made about 4 hours after the worm had been kept at room temperature.

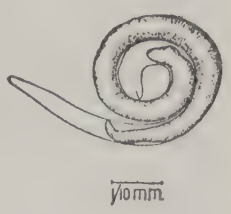

FIG. 2.—*Trichina spiralis:* Outline drawing of a larva in the act of molting outside of the host.

CHANGE IN RESISTANCE OF INTESTINAL TRICHINÆ

A number of investigators have been struck by the marked powers of the intact larvæ to resist various unfavorable conditions. Davaine (*3*) states that he kept larvæ isolated from the muscles alive in fresh water for a month. The same writer also notes that after having reached the adult stage in the intestine this resistance is lost, and that the worms perish in fresh water within one hour.

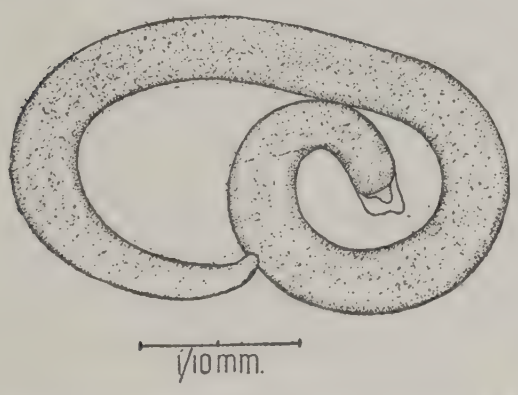

FIG. 3.—*Trichina spiralis:* Outline drawing of a dwarf larva after the first molt outside of the host. The beginning of the second molt is indicated.

The present writer has observed that the decapsuled larvæ are very tolerant to distilled water, concentrated sodium-chlorid solutions, weak solutions of alcohol (35 per cent), potassium cyanid, acids, and many other toxic agents. After the first molt as well as after subsequent molts, and to a lesser extent while the molt is in progress, the tolerance of the larvæ is suddenly reversed and is replaced by a marked sensitiveness. Distilled water is highly toxic to them and brings about their death in a few minutes. The parasites have lost their tolerance for acid, and succumb very rapidly to very weak dilutions (999 cc. of water and 1 cc. of hydrochloric acid, sp. gr. 1.19) This marked sensitiveness character-

izes the intestinal forms at the various stages of their development. The effects of potassium cyanid on the intact larvæ and on the molting forms have been studied especially with a view of determining changes in the resistance of the parasites at various stages of their life history.

These studies have yielded interesting data which the writer expects to present in a separate paper. The poison causes a disintegration in the tissues of the worms without influencing their general outline, due undoubtedly to the cuticle remaining unaffected. Moreover, the worms exhibit a differential sensibility to the poison along the major axis, the wave of disintegration in the intact decapsuled larvæ starting at the anterior end and proceeding posteriorly. When the head end shows a marked paling of the protoplasm accompanied by the beginning of granular degeneration the posterior end of the worm is normal as to color and transparency. The maturing larvæ as well as the sexually ripe specimens taken from the small intestine are not only more susceptible to potassium cyanid and perish almost instantaneously in dilutions in which the decapsuled larvæ survive for several hours, but show a complete reversal of axial susceptibility by being affected in the posterior end, the wave of disintegration gradually moving toward the anterior region. In *Trichinella spiralis* the posterior region consists largely of reproductive cells which, as growth takes place in the small intestine, divide very rapidly and produce thousands of germ cells. In fact, the development of the larvæ in the small intestine is essentially a sexual metamorphosis characterized by a rapid division of the germ cells and the maturation of the latter, accompanied by the appearance of the accessory reproductive organs. It is rather significant that while these changes are taking place in the larvæ the axial susceptibility of the worms to such a depressing agent as potassium cyanid should show the decided reversal described above. In the writer's opinion these facts are a striking confirmation of the views expressed by Child—namely, that there is a direct relation between susceptibility to depressing influences and the degree of metabolic activity.[1]

How can the sudden change in susceptibility of the worms to toxic agents be accounted for? In view of the fact that even the intact larvæ exhibit a differential sensibility to potassium cyanid along their major axis, the cuticle as the all-important factor underlying the resistance of the worms to toxic substances must be eliminated. The fact that the disintegration of the parasites under the influence of potassium cyanid is not haphazard but follows a definite course is direct evidence that the disintegration is a reaction between the tissues and the poison independent of the permeability of the cuticle, and that the rate of reaction varies in different regions of the worm. The reversal of susceptibility in the

[1] Child has published numerous papers dealing with this problem. His views are summarized in two books cited in the list of references (*1*, *2*).

intestina forms is a further confirmation of the view that the tissues of the organism are not passive but take an active part in the reaction. The increased susceptibility of the intestinal worms to potassium cyanid is also probably independent of the cuticle since this increased susceptibility expresses itself in many other ways which are more or less independent of permeability of the membranes of the worms to substances in solution. The loss of tolerance of the intestinal worms to cold, their failure to survive outside of the host for any length of time, their sudden disintegration in salt solution, and the gradual reappearance, though to a markedly lesser degree, of their tolerance to various conditions would indicate that the differences in susceptibility between the intact larvæ and the rapidly developing forms are due to a complete reorganization of the latter as a result of the resumption of differentiation and development.

The encysted larvæ are quite inactive under normal conditions. They neither increase nor decrease in size, but maintain a morphological balance which must have its basis in a delicate adjustment between their nutritive income and their outgo as a result of their processes of oxidation and excretion. With the resumption of growth and further differentiation in the small intestine, the physiological adjustment between income and outgo rapidly breaks down, their constructive metabolism running far ahead of the destructive processes. This leads to an increase in size of the animal and to a differentiation of organs not developed in the encysted larvæ. The intestinal forms in the early stages are thus characterized by a period of rapid growth and may, therefore, be regarded as physiologically younger than the encysted forms. Under the intestinal influences they experience a rejuvenescence, exhibiting a high metabolism which decreases again with age. Parallel with their aging they become more tolerant to adverse conditions. In other words, in the intestine the larvæ recommence a typical life cycle, marked at first by a period of rapid growth and an extreme sensitiveness to unfavorable external conditions, and followed by a period of relative stability and gradually by a reduction in susceptibility to adverse influences.

ATTEMPTS TO INDUCE MOLTING IN THE LARVÆ ARTIFICALLY FREED FROM THEIR CAPSULES

Trichinella spiralis is particularly remarkable in its lack of host specificity. It is capable of reaching maturity in almost any mammalian host and is even able to develop in the intestine of birds. This adaptability to a varied environment would indicate that the factors favorable for its development are the general conditions prevailing in the intestine of warm-blooded vertebrates, such as temperature, food, alkalinity, etc. It therefore appeared to the writer that it would not be a hopeless task to attempt to induce the larvæ isolated from their cysts to molt on artificial media. Thus far all efforts have proved futile, but the work is being

continued with the hope that a medium may be found in which the larvæ will be capable of undergoing the molt that normally occurs a short time after they reach the intestine in the usual course of events when they are swallowed.

In looking through the literature relating to trichinosis the writer has found but scant references to experiments on the possibility of causing the development of the decapsuled larvæ of *Trichinella spiralis in vitro*. Fiedler (*4*) placed pieces of trichinous meat in the gut of a freshly killed rabbit. He tied the gut at both ends and placed it in water at a temperature of 25° to 28° R. Within five days the gut was opened but the trichinæ appeared to be unaffected. Pagenstecher (*5*) states that he did not succeed in causing *T. spiralis* to develop by means of artificial gastric juice. This investigator also placed a portion of intestine containing trichinæ in a sugar solution at body temperature for 24 hours, which resulted in the death of the worms. Davaine (*3*) states that he convinced himself that trichinæ do not develop outside of the host in water or in any other medium.

Among the media tried by the writer the following may be mentioned: Pure blood serum (of rodents), blood serum diluted with salt solution, pancreatin dissolved in an alkaline medium, neutral broth, alkaline broth and various sugar broths, mixtures of pancreatin with various broths, and contents from the small intestine mixed with salt solution. The parasites obtained from trichinous meat by artificial digestion were washed in salt solution and in weak alcohol in order to eliminate bacterial decomposition of the media. The latter were inoculated with the parasites taken up in a sterile pipette with a little sterile salt solution and then placed in an incubator at body temperature. The most favorable results have been obtained with mixtures of blood serum and salt solution and with dextrose broth. In the serum and serum dilutions the larvæ lived considerably longer than when kept in physiological salt solution at incubator temperature, exhibited marked activities, and in one case there were indications of molting. Certain changes in structure were noted which can not be definitely described. The cuticle at the anterior end of the worm had entirely disappeared.

In dextrose broth the larvæ were still alive and active after 24 hours at a temperature of 38° C., and continued alive for two days at room temperature, whereas in plain broth and in other sugar broths, as well as in neutral media, such as various salt solutions, they died before 24 hours had elapsed. In one case larvæ kept in an alkaline pancreatin solution were found to exhibit unusual acivity, including intestinal peristalsis.

SUMMARY AND CONCLUSIONS

(1) The larvæ of *Trichinella spiralis* do not linger in the stomach of the host after they are freed from their capsules, but pass into the small intestine.

(2) The passage of the larvæ through the stomach does not stimulate them to further growth and development, and a brief sojourn in the intestine is insufficient to initiate those processes which lead to sexual maturity.

(3) Larvæ from the intestine that have not yet been stimulated to further development become tightly coiled when removed from the host and placed in a physiological salt solution, but those which have been stimulated to development apparently lose the power of becoming tightly coiled under similar conditions.

(4) Larvæ which have been stimulated to further development in the intestine will molt even after being removed from that organ. The molting process may be hastened by high temperatures and suppressed by low temperatures.

(5) Larvæ which have not yet been stimulated to further development in the small intestine can not be caused to molt by a high temperature.

(6) With the beginning of development in the small intestine the larvæ lose the power of surviving for considerable lengths of time outside of the host. They afterward become more persistent, however, in direct proportion to their increasing age.

(7) When removed from the host within 24 hours after artificial infection intestinal trichinæ often undergo spontaneous disintegration which may be due to the sudden change of environment, lack of food, or possibly the liberation of toxic substances which affect the parasites while in an artificial medium.

(8) Larvæ which molt after removal from the host have been observed occasionally to decrease in size. It is suggested that the dwarfed condition is possibly due to lack of food.

(9) After the first and subsequent molts the tolerance of the larvæ to various toxic agents is replaced by a marked sensitiveness to such agents which decreases, however, with advancing age.

(10) Under the influence of potassium cyanid the worms undergo disintegration and exhibit susceptibility to the poison along the major axis which in the growing forms appears to be greatest in regions where growth takes place most rapidly.

(11) Modifications in the permeability of the cuticle do not appear to be directly responsible for the changes in susceptibility. The changes probably result from a reorganization of the protoplasm coincident with growth, differentiation, and age.

(12) Attempts to induce molting in the larvæ which have been decapsuled by artificial digestion and afterwards kept *in vitro* under various conditions have thus far failed to yield successful results.

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EXPERIMENTS ON THE VALUE OF GREENSAND AS A SOURCE OF POTASSIUM FOR PLANT CULTURE

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HISTORICAL INTRODUCTION

The practice of applying to farm lands deposits known under the broad term "marl" has a long history both in Europe and in America. Among those deposits occurring in the Atlantic coastal region from New Jersey to Virginia are the so-called "greensands" which soon after the American Revolution came into extensive use as fertilizers. These greensands were sometimes found as relatively pure glauconite, but perhaps oftener contained more or less abundant quantities of fossil shells. The lime component was frequently sufficient to give to these deposits the character of real marls.

Analyses of New Jersey greensands made by Seybert [1] in 1822 showed that iron, silica, and potash are present in pure greensand. Samples of greensand marl were found to contain in addition varying quantities of lime, phosphates, and other constituents. The value of these deposits as fertilizers was variously ascribed by different investigators to the lime, the iron, and the phosphates present. Since, however, it was not definitely determined until the early sixties that potassium is necessary for plant growth, the presence of this element could be only suspected to have connection with the valuable properties observed.

That great gains were seen in crops following the application of greensands and of greensand marls was the usual experience, and the practice of "marling" during the quiet winter months became the rule in those parts of New Jersey and Virginia in which the deposits were easy of access. This practice grew to very great proportions in a number of regions, railroads even being built in New Jersey to haul marl from pits yielding a product of high reputation out into the adjacent country.

The digging and carting of these heavy deposits, however, was a severe drain on labor, and when in the sixties concentrated soluble

[1] SEYBERT, Henry. ANALYSIS OF THE GREEN EARTH FROM RANCOCAS CREEK, NEW JERSEY. *In* Mem. Phila. Soc. Prom. Agr., v. 5, p. 21. 1826.

fertilizers were introduced the use of these marls quickly fell off. At the present day they are practically neglected.

DISTRIBUTION AND NATURE OF GREENSAND DEPOSITS

These deposits occur most abundantly in the Atlantic coastal plain lying on the seaward side of the "fall line," where they are oftentimes extensively exposed by the rivers which cut this line. The most important greensand deposits of the eastern States extend from Navesink Heights near Red Bank, N. J., in a southwesterly direction to a region below the James River southeast of Richmond.

The materials vary in appearance from greenish black when the greensand is fairly pure to a grayish color, which is determined by the proportion of shells and sand present. Greensand (glauconite) can usually be demonstrated as small rounded grains, black or greenish in color, present in the earth embedding the shells as a matrix and filling the hollows of the shells. The grains leave a green streak when rubbed on hard paper with a knife blade.

Especially prominent in many Virginia deposits is an overlying layer of somewhat different character, called by Edmund Ruffin "olive earth." This material, while not a greensand deposit, must be considered practically as a part of it, since it must usually be dealt with before the underlying greensand deposits can be reached. These olive earths frequently contain sufficient quantities of calcium carbonate and phosphates to give them considerable value.

CHEMICAL COMPOSITION OF GREENSAND DEPOSITS

Typical deposits from both New Jersey and Virginia were collected for this work and analyzed with special reference to the constituents generally recognized as having major importance for plant growth.[1] The materials used in the culture experiments reported below gave the results seen in Table I.

[1] Mr. Otis F. Black, Chemical Biologist, and Mr. James W. Kelly, Chemical Technician, of this Office, made the analyses.

TABLE I.—*Analyses of greensand deposits used in experiments*

Material.	Calcium oxid.	Phosphorus pentoxid.	Sulphur trioxid.	Potassium oxid.	
				Soluble in dilute hydrochloric acid.	Total potassium oxid present.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1. Greensand, Courtland, Hanover County, Va.	0.33	0.16	0.27	2.65	5.76
2. Greensand, Red Bank, Monmouth County, N. J.	1.75	.95	Trace.	6.80	7.63
3. Greensand marl, Red Bank, Monmouth County, N. J.	7.45	1.25	Absent.	6.10	6.77
4. Greensand marl, Hanover County, Va.	12.50	.17	.50	1.85	2.16
5. Greensand marl, near Tunstall, New Kent County, Va.	9.20	.33	.26	1.35	1.52
6. Greensand marl, mixture of equal parts of No. 4 and 5	10.85	.25	.38	1.60	1.84

EXPERIMENTAL METHODS

The purpose of the experimental work reported here was to ascertain whether these deposits are capable of serving as a useful source of potassium for growing plants, and if so, how promptly the potassium becomes available. It is shown clearly in the above analyses and in scores of others to be found in the extensive literature that potassium is present in greensands in considerable quantity, but earlier investigations tended to show that it is present in the form of a rather stable silicate the availability of which for plant uses has been called in question.[1]

In order to gain time and the better to control conditions of work, pot experiments were carried out in the greenhouses of the department during the past winter (1917–18). Glazed earthen pots of the ordinary 6-inch type were first used, but it was found that the glazing was usually checked by many fine cracks which reached through the surface of the glaze. The pots themselves were found to furnish soluble impurities, among them potassium compounds, in such quantities as led to the abandonment of this type of container. Common porous pots, well soaked in hot paraffin, the surplus of which was quickly drained off while still hot, gave satisfactory results. The saucers were treated in the same way.

The purest obtainable sand was used as a solid medium. A grade known as "crushed quartz" was found to be satisfactory when submitted to rather searching tests.

The best chemicals were used in making up the culture solutions.

In view of the fact that the problem was expected to give results of practical value, wheat (*Triticum aestivum*) and red clover (*Trifolium pratense*) were chosen as suitable test plants.

[1] It should always be borne in mind in considering problems of this nature that the plant's operations are not bounded by the limits which prevail in chemical-laboratory tests, and reactions that are difficult in the test tube are sometimes easily performed by the living plant.

As a source for the necessary nutrient salts a formula of culture solution worked out by Shive [1] for wheat was made use of. Since no similarly tested formula for clover culture was available, the same solution was used for the clover experiments. Shive's solution (R_5C_2) was made up on the following basis:

Potassium phosphate (KH_2PO_4).....	0.0180 mole in 1 liter of solution.
Calcium nitrate [$Ca(NO_3)_2$]	.0052 mole in 1 liter of solution.
Magnesium sulphate ($MgSO_4$).....	.0150 mole in 1 liter of solution.

All salts are calculated as water-free.

In this solution we should expect to have whatever mineral materials are necessary for wheat plants in proportion and concentration found by culture test to be most fitting.

To get a standard with which to compare the results obtained with the marls, a series of culture solutions was made up on the basis of Shive's solution so modified as to supply the potassium in the form of the common salts of commerce. These in very impure form have furnished a large part of the potassium present in the commercial fertilizers of the past and should give results up to which marls should measure if they are to be substituted for these salts as equally satisfactory sources of agricultural potassium.

It is well known that potassium absorption is greatest during the time when increase in volume is the predominant feature of plant development; hence, chiefly during the earlier stages of the life of the plants. The experiments, therefore, were not continued to maturity, the crop being harvested and dried for comparison after a growth period of eight weeks from the planting of the seed.

The pots, nearly filled with sand, were well watered with the desired culture solutions in case all constituents were soluble, and the seeds of wheat or red clover were immediately planted. The use of a surplus of solution was avoided in order that materials might not be washed down into the saucers and beyond the reach of the plants.

In the case of the slowly soluble greensands the quantity of material needed was weighed out and thoroughly mixed with the dry sand. These mixtures were put into the pots and irrigated with culture solutions from which all sources of potassium were omitted. It was hoped in this way to find out whether the greensand materials could yield useful potassium rapidly enough to permit the plants to make growth. Control cultures in which all nutrients but potassium were present gave a measure of the gain resulting from the presence of the salt or of the greensand. Other controls with complete culture solutions, which would be expected to yield the most favorable results were provided.

[1] SHIVE, John W. A THREE-SALT NUTRIENT SOLUTION FOR PLANTS. *In* Amer. Jour. Bot., v. 2, no. 4, p. 159. 1915. (p. 157–160.)

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RESULTS WITH POTASSIUM SALTS

Culture solutions containing potassium, whether supplied in the form of the common salts or as greensands or marls, were made up on the basis of the number of pounds of potassium supplied to a volume of soil having a surface of 1 acre and a depth of 1 foot. In the case of the slowly soluble deposits this gives a measure of direct applicability. For the salt solutions the relation is less usual, but is still a practicable way of calculating concentration. The potassium content in each case was calculated on the quantity known by analysis or formula to be present, solubility not being considered.

In Table II are given data showing the growth made by Turkey Red wheat and red clover in a series of pot sand cultures in which the potassium demand was supplied by the common salts. The concentration of potassium in each culture is given in the number of pounds applied per acre-foot. The weights of wheat tops and red-clover tops in an air-dry condition are given in other columns. The plants were harvested eight weeks after planting (see Pl. 33).

TABLE II.—*Results of the growth of wheat and red clover in quartz-sand cultures, with potassium supplied by common soluble salts*

Quartz sand plus potassium sources.	Potassium (pounds per acre).	Wheat tops (air-dry).	Red-clover tops (air-dry).
		Gm.	*Gm.*
Control:			
Culture solution (no potassium)	0.0	0.75	0.43
Shive solution complete	103.7	2.09	2.84
Potassium nitrate (KNO_3)	88.6	3.22	2.71
Do	433.2	2.62	1.75
Do	866.4	2.21	1.21
Do	1,772.8	2.10	.25
Do	2,659.3	.84	.00
Total		10.99	5.92
Potassium phosphate (KH_2PO_4)	88.6	1.95	1.80
Do	433.2	1.37	1.88
Do	866.4	1.44	.80
Do	1,772.8	.92	.20
Do	2,659.3	.25	.00
Total		5.93	4.68
Potassium chlorid (KCl)	88.6	1.35	1.00
Do	433.2	1.35	.59
Do	866.4	1.65	.69
Do	1,772.8	1.20	.63
Do	2,659.3	.97	.32
Total		6.52	3.23
Potassium sulphate (K_2SO_4)	88.6	1.10	.66
Do	433.2	1.53	.70
Do	866.4	1.36	1.03
Do	1,772.8	1.17	.50
Do	2,659.3	.95	.05
Total		6.11	2.94

In the control solutions, a rough idea of the growth that can be made on the reserve potassium stored in the seeds is shown in the first line, in which sand and a nutrient solution containing no potassium were present. In the second line the development made in the complete Shive culture solution gives what should be a maximum result. It is but fair to point out that in working out this formula Shive used wheat, for which it was the most favorable combination he obtained. It is quite possible that red clover might prosper better in a somewhat different combination. Since, however, no carefully worked out data, such as Shive has given for wheat, were known for clover, it was decided to use the same solution for both plants in the belief that this same solution would be favorable, though perhaps not the most favorable that might be found.

As a general outstanding feature of this series it will be noted that growth decreases as concentration increases. This seems to indicate that the concentrations lie between the maximum and the toxic, contrasting sharply, as will be seen, with the slowly soluble marls and greensands. A number of cases seem to call for comment.

For both the potassium-nitrate and potassium-phosphate series it seems likely that the minimum concentration of 88.6 pounds of potassium per acre is the most favorable one offered for wheat, the increase in quantity of salt being accompanied by a decreasing growth. In the case of the clover 433.2 pounds of potassium, offered as potassium phosphate, is a little more favorable than 88.6 pounds, indicating that neither of these quantities is injuriously high.

It may be noted that in all nitrate cultures except the most concentrated the growth of the Shive control is equaled or exceeded, whereas in no clover culture was the control quite equaled.

In the case of potassium chlorid and potassium sulphate a somewhat different result appears. In no culture with either wheat or clover containing either salt was the growth made in the Shive control equaled. A further point of interest lies in the fact that the maximum growth of wheat was found in higher concentrations, 866.4 pounds of potassium in potassium chlorid and 433.2 pounds in potassium sulphate, indicating that the most favorable concentration lies somewhere in the range here covered and that reduced growth in the less concentrated members of the series is not due to harmful concentration of salts. In the case of the clover the best result with potassium chlorid is seen in the greatest dilution, and a more favorable result still might have been gained had a still smaller quantity of this salt been used. In the case of potassium sulphate the best result is seen in higher concentration, 866.4 pounds per acre.

Since the two latter salts have a greater commercial significance in connection with the commercial potassium supply, it is of especial importance to note the results following their use.

It is clear that potassium nitrate is able to supply something that gives this salt an advantage clearly not derived from the potassium content solely. It is probable that the nitrate ion contributed a favorable action lacking to the other potassium salts used.

As a practical means of meeting the potassium need this could hardly be considered a practicable resource under present conditions.

RESULTS WITH GREENSANDS AND GREENSAND MARLS

A chemical study of the composition of greensand deposits shows a wide variation not only in the materials present but likewise presents almost every possible combination in proportions present. While potassium, silica, and iron are ever-present constituents of glauconite, with it are oftentimes found sulphates and phosphates, the latter sometimes in considerable quantity. Calcium, usually present, is, of course, a major constituent in the marl types.

It is obviously difficult to get a strict basis of comparison between these complex mixtures of slowly-soluble materials and the readily-soluble pure salts above reported. It was hoped, however, that this might be done by supplying in the culture solutions with which the pots containing these sand and marl mixtures were watered sufficient phosphates and sulphates to supply the necessary demand of the rather sparse plantings made in the pots. Such necessary constituents present in quantities sure to be sufficient to satisfy the demands of the plants would tend to reduce or efface the influence of additional quantities of these substances added in the marls. The only required constituent not present in the basic culture solution would be potassium, which would of necessity be drawn by the plants from the marls or greensand, if they were to get them anywhere.

After deposits from several regions, chiefly from Virginia and New Jersey, had been collected and analyzed, certain typical deposits from both States were selected for use in these experiments. Two samples called "greensand," because of the low lime content were selected, one from each State. Two samples of "greensand marl," containing from 7.45 to 12.50 per cent of calcium oxid were also used, one from New Jersey and a mixture of equal parts of two from Virginia. All are low in phosphates and sulphates and vary widely in their potassium content.

In making up the cultures in the manner already described, the quantities of marl taken cover the rates of application customary in the days of "marling." The potassium present was calculated, not on the more readily available portion extracted with dilute hydrochloric acid but on the total potassium present, determined by the hydrofluoric- acid method.[1]

[1] HICKS, William B., and BAILEY, Reginald K. METHODS OF ANALYSIS OF GREENSAND. *In* U. S. Geol. Survey Bul. 660–B, p. 53. 1917.

In Table III appear the results obtained with wheat and red clover recorded as in Table I (see Pl. 34).

TABLE III.—*Results of the growth of wheat and red clover in quartz-sand cultures, with potassium supplied by greensand and greensand marls*

Quartz sand plus potassium sources.	Potassium in pounds per acre.	Wheat tops (air dry).	Red clover tops (air dry).
		Gm.	*Gm.*
Culture solution (no potassium)	0.0	0.75	0.43
Shive solution complete	103.7	2.09	2.84
Courtland, Va., greensand:			
1 ton per acre-foot	88.6	1.23	.85
5 tons per acre-foot	433.2	1.14	.68
10 tons per acre-foot	866.4	.40	.24
20 tons per acre-foot	1,772.8	.22	Dead.
30 tons per acre-foot	2,659.3	.21	Dead.
Total	.....	3.20	1.77
Pamunkey Valley, Va., shell marl:			
1 ton per acre-foot	30.5	1.33	1.10
5 tons per acre-foot	152.5	1.79	1.37
10 tons per acre-foot	305.0	1.78	2.05
20 tons per acre-foot	610.0	1.95	1.82
30 tons per acre-foot	915.0	2.12	1.87
Total	.....	8.97	8.21
Control (no potassium)	.0	.96	.47
Shive solution complete	103.7	1.98	1.49
Red Bank, N. J., greensand, lower layer:			
1 ton per acre-foot	126.6	1.59	1.10
5 tons per acre-foot	633.0	1.95	2.60
10 tons per acre-foot	1,266.0	2.00	1.90
20 tons per acre-foot	2,532.0	2.27	1.60
30 tons per acre-foot	3,798.0	2.02	1.52
Total	.....	10.83	8.72
Red Bank, N. J., marl, upper layer:			
1 ton per acre-foot	112.3	1.00	.82
5 tons per acre-foot	561.0	1.62	1.36
10 tons per acre-foot	1,123.0	1.96	1.87
20 tons per acre-foot	2,246.0	1.89	1.67
30 tons per acre-foot	3,369.0	2.07	2.10
Total	.....	8.54	9.82

The work reported in Table III was carried out in two series, the Courtland greensand and Pamunkey shell marl with their controls, preceding the experiment with the two Red Bank deposits and their controls. It is believed, however, that all may be compared, since the surrounding conditions were subject to no significant variation.

It will be noted that, owing to the widely varying potassium content of these deposits, the same tonnage applied gave widely different quanti-

ties of potassium. These values are indicated as before in terms of pounds of potassium per acre-foot.

On recalling the experiments with potassium-containing salts (Table II), it will be remembered that the greatest weight of tops was usually seen in the weaker solutions, falling off as the concentration was increased. This was regarded as being due to the harmful action of the excess salts.

Here the case is in general the opposite, the weight of tops increasing as the quantity of marl or of greensand increases. This may be interpreted as being due to the increasing quantity of potassium obtained by the plants from the greater surface of the potassium-yielding particles. It seems probable that in all cases except in that of the Courtland greensand the potassium demand is fully satisfied in the cultures containing the maximum quantity of both greensand and marl, in all three cases the plants approaching or exceeding in growth the corresponding control in Shive's complete solution.

It will be further noted that even where smaller doses of marl are used (1 to 5 tons per acre-foot) the yield often exceeds the result obtained with potassium salts in their most favorable concentration. Thus the poorest yield with Red Bank greensand (1.59 gm. of tops with an application of 1 ton per acre-foot, containing 126 pounds of potassium) exceeds the yield obtained with potassium sulphate (1.53 gm. with 433.2 pounds of the potassium per acre-foot), and almost equals the best yield with potassium chlorid (1.65 gm. with 866.4 pounds per acre-foot).

In all cases (Courtland greensand excluded) the best results with marls and greensands exceed the best results with potassium salts (potassium nitrate excepted).

A comparison of the total growths made in each series of cultures helps to give an idea of the value of each material in the quantities here used. Such a summary is given in Table IV.

TABLE IV.—*Total weights of wheat and red clover in potassium salts and greensand marl*

Material.	Potassium salts—Air-dry tops.		Material.	Greensand marl—Air-dry tops.	
	Wheat.	Red clover.		Wheat.	Red clover.
	Gm.	*Gm.*		*Gm.*	*Gm.*
Potassium nitrate	10.99	5.92	Courtland, Va., greensand	3.60	1.77
Potassium phosphate	5.93	4.68	Red Bank, N.J., greensand	10.83	8.72
Potassium chlorid	6.52	3.23	Pamunkey Valley, Va., marl	8.97	8.21
Potassium sulphate	6.11	2.94	Red Bank, N. J., marl	8.54	9.82

While the growth made in cultures in which the potassium demand is satisfied by greensand deposits (the poisonous Courtland greensand excepted) is markedly superior to that contained in cultures containing the commoner potassium salts (potassium nitrate excepted), it must be remembered that in the case of the salts a majority of the cultures contain an excess of salts, whereas in the marl and greensand cultures the concentration rarely exceeds the optimum and probably still less often attains it.

Concerning Courtland greensand, so often excepted in the above discussion, it may be said that this deposit has been found to belong to the unusual class of so-called "poisonous marls." Since this is a very unusual case and one not likely to be often encountered, it is sufficient to say that ground limestone added at the rate of a ton or more to the acre seems likely to render this greensand a useful fertilizer when applied at the rate of 1 to 5 tons per acre.

CONCLUSIONS

It has been shown by pot experiments carried out with crushed quartz and Shive's culture solution as a basis that greensands and greensand marls from Virginia and New Jersey are able to supply sufficient potassium to satisfy the demand of Turkey Red wheat and red clover during the first two months of their growth. This enables them to make a greater dry weight of tops than was seen in similar cultures in which the potassium demand was supplied by potassium chlorid, potassium sulphate, and potassium phosphate. The prompt availability of sufficient potassium to meet the needs of many, perhaps most, farm crops seems to be indicated.

PLATE 33

Sand cultures with potassium salts:

A.—I, Turkey Red wheat. Potassium supplied in potassium nitrate.

1, Control in culture solution containing no potassium.

2, Control culture in complete Shive solution (R_5C_2).

3, Culture solution, 88.6 pounds of potassium per acre.

4, Culture solution, 433.2 pounds of potassium per acre.

5, Culture solution, 866.4 pounds of potassium per acre.

6, Culture solution, 1,772.8 pounds of potassium per acre.

7, Culture solution, 2,659.3 pounds of potassium per acre.

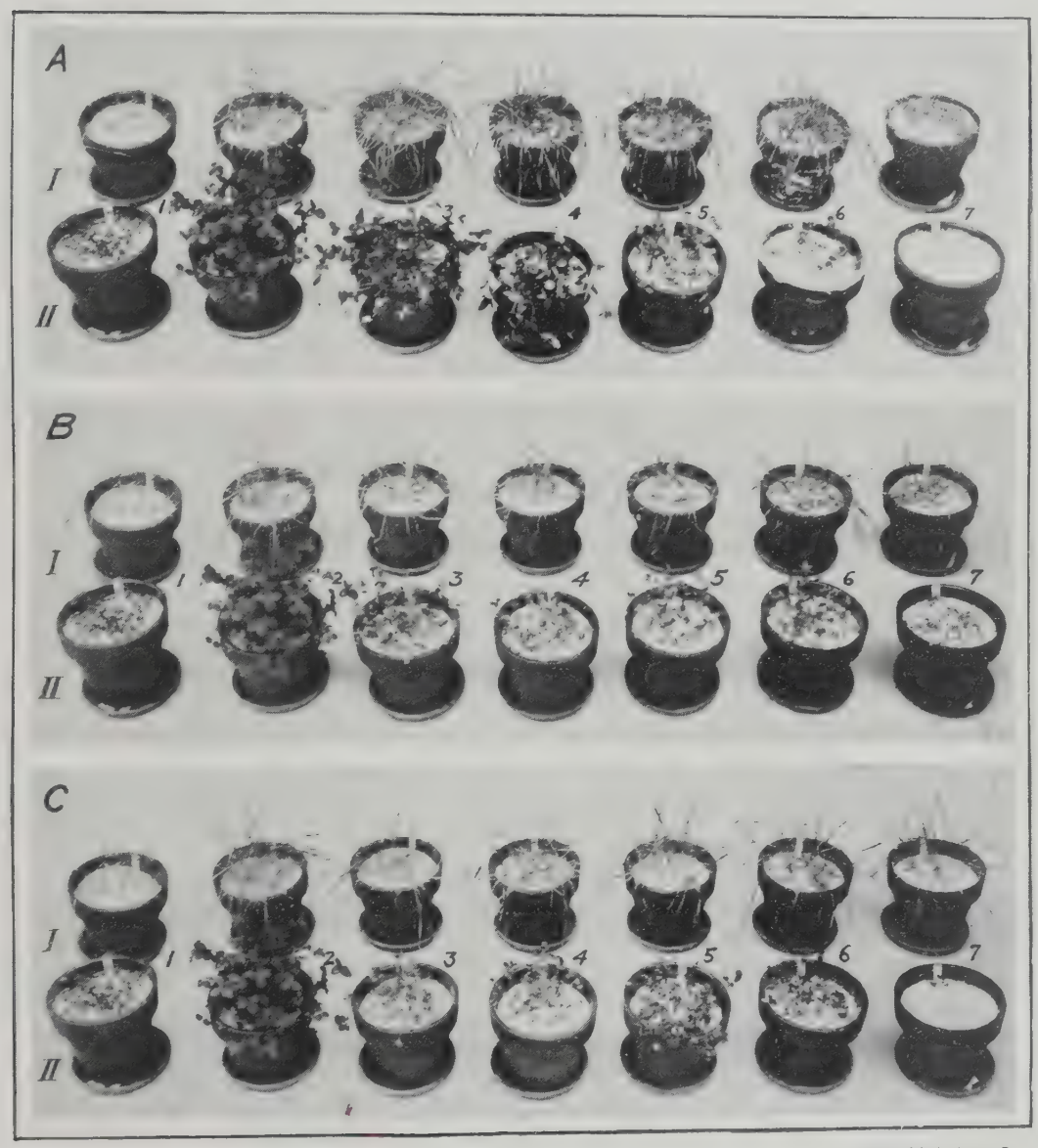
II, Red clover. Potassium supplied in potassium nitrate. Individual cultures as above indicated.

B. — I, Turkey Red wheat. Potassium supplied in potassium chlorid. Individual cultures as above indicated.

II, Red clover. Potassium supplied in potassium chlorid. Individual cultures as above indicated.

C. — I, Turkey Red wheat. Potassium supplied in potassium sulphate.

II, Red clover. Potassium supplied in potassium sulphate. Individual cultures as above indicated.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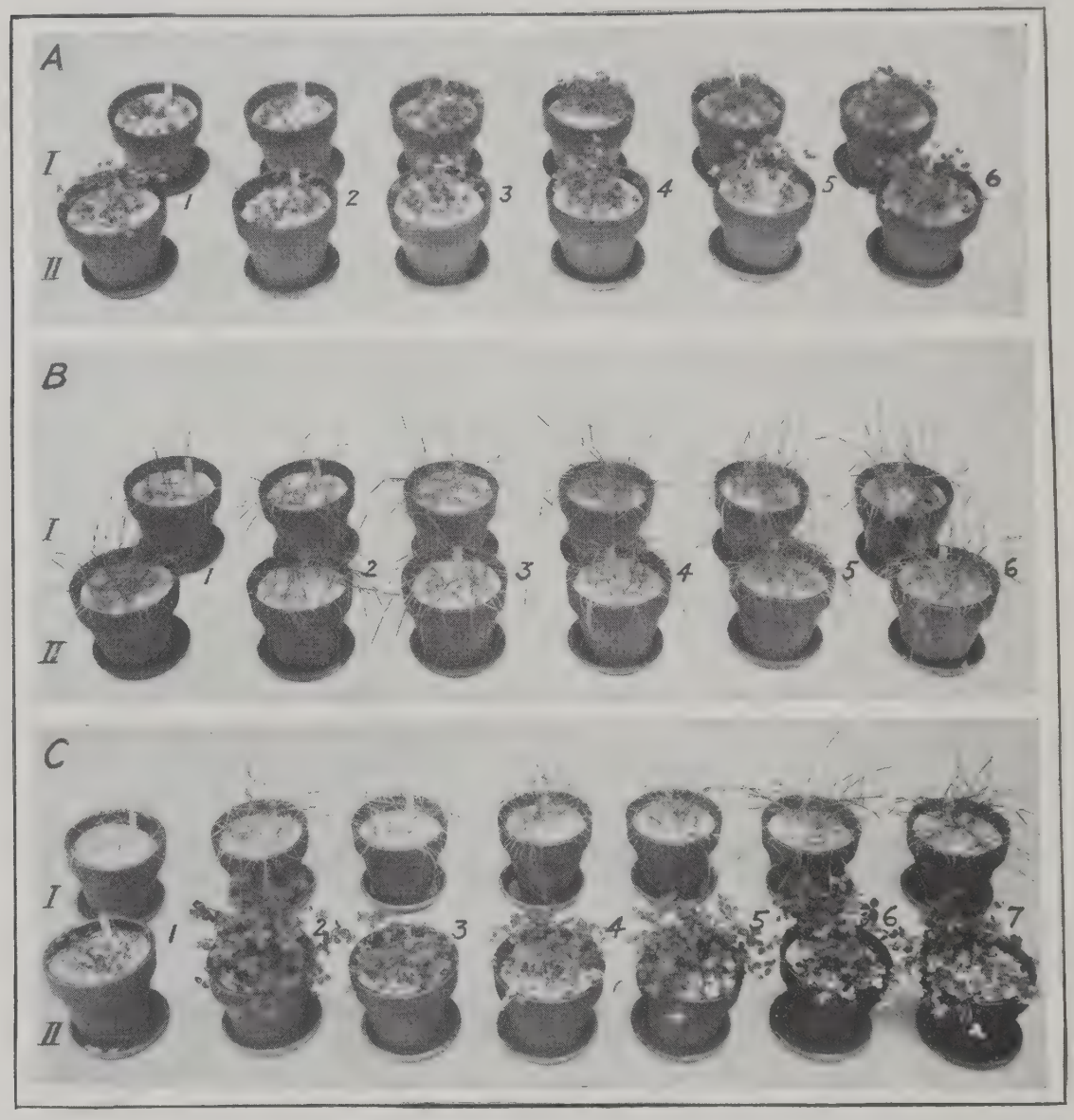
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PLATE 34

Cultures with greensand deposits:

A.—Red clover. Potassium supplied in greensand deposits from Red Bank, N.
I, Series with greensand marl from upper stratum.
II, Series with greensand from lower stratum.
I—1, Control culture solution containing no potassium.
II—1, Control culture. Complete Shive solution (R_5C_2).
I—2, Culture solution lacking potassium, plus 1 ton of greensand
per acre-foot.
II—2, Culture solution lacking potassium, plus 1 ton of greensand
acre-foot.
I—3, Culture solution lacking potassium, plus 5 tons of greensand
per acre-foot.
II—3, Culture solution lacking potassium, plus 5 tons of greensand
acre-foot.
I—4, Culture solution lacking potassium, plus 10 tons of greens
marl per acre-foot.
II—4, Culture solution lacking potassium, plus 20 tons of greensand
acre-foot.
I—5, Culture solution lacking potassium, plus 20 tons of greensand
per acre-foot.
II—5, Culture solution lacking potassium, plus 20 tons of greensand
acre-foot.
I—6, Culture solution lacking potassium, plus 30 tons of greensand
per acre-foot.
II—6, Culture solution lacking potassium, plus 30 tons of greensand
acre-foot.

B.—Turkey Red wheat. Potassium supplied in greensand deposits from
Bank, N. J. Designations of series and of individual cultures as above.

C.— I, Turkey Red wheat. Potassium supplied in greensand marl from
munkey Valley, Va.
1, Control. Culture solution containing no potassium.
2, Shive solution, complete.
3, Culture solution lacking potassium, plus 1 ton of greensand marl
acre-foot.
4, Culture solution lacking potassium, plus 5 tons of greensand marl
acre-foot.
5, Culture solution lacking potassium, plus 10 tons of greensand
per acre-foot.
6, Culture solution lacking potassium, plus 20 tons of greensand
per acre-foot.
7, Culture solution lacking potassium, plus 30 tons of greensand
per acre-foot.
II, Red clover. Potassium supplied in greensand marl from Pamunkey
ley, Va. Designations of individual cultures as in C–I.

EFFECT OF FARM MANURE IN STIMULATING THE YIELDS OF IRRIGATED FIELD CROPS

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LOCATION OF THE EXPERIMENTS

The experiments reported in this paper were conducted at three field stations operated by the Bureau of Plant Industry. These stations are all located in the northern Great Plains and fairly represent conditions in that portion of the area lying in the eastern parts of Wyoming and Montana and in the western parts of Nebraska and South Dakota.

The three stations are as follows: (1) The Scottsbluff Station,[1] located on the North Platte Reclamation Project, north of Scottsbluff and east of Mitchell, Nebr.; (2) the Belle Fourche Station, located on the Belle Fourche Reclamation Project, about 30 miles east of Belle Fourche, near the town of Newell, S. Dak.; (3) the Huntley Station,[2] located on the Huntley Reclamation Project about 20 miles east of Billings, near the town of Huntley, Mont.

Each of these stations represents a large section of irrigated land, not only that included within the limits of individual reclamation projects but even more extended areas which are adjacent in the same drainage basins. The climate of the whole section is essentially semiarid in character, with an average annual precipitation of about 14 inches, most of which occurs in the spring and early summer, and with a frost-free growing season of about 125 days. The altitude of Scottsbluff is 4,000 feet, while Belle Fourche and Huntley are both 3,000 feet above sea level.

AGRICULTURAL AND SOIL CONDITIONS OF THE REGION

The agriculture of the region is relatively new, there having been very little crop production prior to 1890, and the present era of development may be said to have begun about 1900. During the 30 years or more preceding the beginning of crop production, the region was utilized chiefly as range land for cattle and horses. In recent years, despite the extension of dry-farming and irrigation, the use of the range lands for live-stock production has continued, and this industry remains an important one throughout the region.

[1] The work of this field station is conducted and supported cooperatively by the United States Department of Agriculture and the Nebraska State Experiment Station.

[2] The work at this field station is conducted and supported cooperatively by the United States Department of Agriculture and the Montana State Experiment Station.

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The more important crops of irrigated lands of the region are alfalfa (*Medicago sativa*), cereals, including corn (*Zea mays*), oats (*Avena sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum* spp.), sugar beets (*Beta vulgaris*), and Irish potatoes (*Solanum tuberosum*). On the three reclamation projects referred to above, comprising 150,000 acres, these crops occupy nearly 90 per cent of the land, in the following proportions: Alfalfa, 43 per cent; cereals, 31 per cent; sugar beets, 10 per cent, and potatoes, 3 per cent.

The soils are generally very productive, and, notwithstanding the hazards of occasional hail storms and of the occurrence of insect pests and plant diseases, abundant crops are produced. The North Platte Project, which includes the Scottsbluff Station, has a light sandy soil which takes water readily and is subject to wind erosion when left exposed without a plant cover. The Belle Fourche soil is a heavy black clay, very plastic when wet, becoming friable on drying, when large shrinkage cracks are formed through which water penetrates the dry soil rapidly. The soil at Huntley is not quite so heavy as that at Belle Fourche, though it would be classed as a clay loam. It is very productive, except where the salt content is too high.

DESCRIPTION OF THE EXPERIMENTS

The experiments here discussed are a part of a large series of rotation experiments conducted under irrigation at each of the three stations. These rotation experiments were started with the crop season of 1912 and have been continued without modification. The field plots include ¼ acre each. These are laid out in series which are separated by 40-foot roads, and the plots in the series are separated by 5-foot alleys. The plots at Scottsbluff are 132 feet long by 82.5 feet wide. Those at Belle Fourche are 264 feet long by 41.25 feet wide, and those at Huntley are 227 feet long by 48 feet wide.

The present paper deals only with the effect of manure on the yields and qualities of two crops, sugar beets and potatoes, at the three stations for the 6-year period 1912 to 1917. The results are based on five pairs of rotations, the two members of each pair differing from each other only in that farm manure is applied annually, at the rate of 12 tons per acre, to one crop in one rotation, and no manure is applied to the other rotation. Each rotation occupies as many plots as there are years in the cycle, so that each crop is represented each year. The crops and sequences involved in the 10 rotations are as follows:

Rotation 20: Potatoes; sugar beets.
Rotation 21: Potatoes (manured); sugar beets.
Rotation 22: Oats; sugar beets.
Rotation 23: Oats; sugar beets (manured).
Rotation 24: Oats; potatoes.
Rotation 25: Oats; potatoes (manured).
Rotation 30: Potatoes; oats; sugar beets.
Rotation 31: Potatoes; oats; sugar beets (manured).
Rotation 60: Potatoes; oats; sugar beets; alfalfa, first year; alfalfa, second year; alfalfa, third year.
Rotation 61: Potatoes; oats; sugar beets (manured); alfalfa, first year; alfalfa, second year; alfalfa, third year.

With respect to any given plot the same crop recurs with each cycle of the rotation. Thus in the six years since 1912 the crops in the 2-year rotations have been on the same plot three times, those in the 3-year rotations have been on the same plot twice, while in the 6-year rotations only one cycle has been completed. In the case of the 2-year and 3-year rotations, in which manure is applied to one of the crops, this manured crop has received each year the immediate benefit of the manure and in addition the benefit of any residual effect that may have remained from the earlier applications. This becomes true also in the 6-year rotations after the completion of the first cycle.

One pair of the 2-year rotations and the pair of 3-year rotations include both sugar beets and potatoes. In the 2-year rotation, No. 21, the manure is applied preceding the potatoes and the beet crop that follows gets only the second-year residual effect of the manure. In the 3-year rotation, the manure is applied preceding the sugar beets and the potatoes receive the second-year residual effect. In the other rotations the effect of the manure is noted only in respect to the crop which immediately follows its application.

The cultural operations used with these rotations are only such as ordinary good farming demands. So far as the rotation pairs are concerned, all cultural operations are the same for both members of the pair. The same varieties of the two crops are used in all rotations at each station each year. The same varieties are not used at the different stations; nor has the same variety been used for all years at each station. For the sugar beets the seed has usually been obtained from the local sugar factory, and for the potatoes some good locally adapted variety has been used.

The field work of these rotation experiments has been under the direction of the farm superintendent of each station, and under the immediate supervision of a scientific assistant, who is charged with performing or directing the cultural operations, the irrigation, the harvesting, and taking the field notes and reporting the results each year.[1]

RESULTS OF THE EXPERIMENTS

IRISH POTATOES

The effect of the manure on the yield of potatoes is shown in Table I, which gives the yields in bushels per acre for the manured plots and the yields from plots that have had similar treatment, except for the manuring.

[1] The following is the personnel concerned with the field work of these rotation experiments: At Scottsbluff Mr. Fritz Knorr was superintendent of the field station from 1910 to the end of 1916. Mr. James A. Holden was in charge of the irrigated rotations at this station from 1912 to the end of 1916, when he succeeded Mr. Knorr as station superintendent. Mr. David W. Jones supervised the irrigated rotations during 1917.

At Bellefourche Mr. Beyer Aune has been superintendent of the field station since 1909. He has been closely in touch with the irrigated rotation work, being assisted in it at different times by Mr. John B. Wentz, Mr. N. L. Mattice, and Mr. George T. Ratliffe.

At Huntley Mr. Dan Hansen has been superintendent of the field station since 1910. The irrigated rotations were under the supervision of Mr. John M. Spain during 1912, Mr. John W. Knorr during 1914 and Mr. Edward G. Noble since 1915.

In rotations 21 and 25 manure was applied each year preceding the potato crop, while in rotation 31 the manure was applied for the sugar-beet crop which preceded the potatoes. The annual differences in yield between the manured and unmanured plots are also shown in the tables, together with the mean annual yield of the crop in each rotation and the mean of the annual differences in yield. With each of the means the probable error is given.[1]

TABLE I.—*Effect of manure on the yields of Irish potatoes at the Scottsbluff, Nebr., Belle Fourche, S. Dak., and Huntley, Mont., field stations, 1912 to 1917*

[The yields, differences, and means are expressed in bushels per acre]

SCOTTSBLUFF

Rotation No.	1912	1913	1914	1915	1916	1917	Mean.
20 (no manure)	194	398	146	88	142	131	183±28.4
21 (manure)	230	316	237	147	155	187	212±18.5
Difference	+36	−82	+91	+59	+13	+56	+29±16.0
24 (no manure)	252	235	146	109	217	134	182±19.9
25 (manure)	230	348	253	148	216	182	229±18.0
Difference	−22	+113	+107	+39	−1	+48	+47±15.9
30 (no manure)	268	329	216	146	226	167	225±18.5
31 (manure)	[a] 250	353	243	175	244	211	246±13.9
Difference	−18	+24	+27	+29	+18	+44	+21± 5.6

BELLE FOURCHE

20 (no manure)	71	128	86	102	157	133	113±10.0
21 (manure)	59	133	117	192	170	190	144±15.3
Difference	−12	+5	+31	+90	+13	+57	+31±10.8
24 (no manure)	13	109	112	111	151	120	103±11.0
25 (manure)	41	95	101	168	162	135	117±14.1
Difference	+28	−14	−11	+57	+11	+15	+14± 7.2
30 (no manure)	50	74	68	59	166	139	93±15.1
31 (manure)	[a] 54	90	140	137	188	205	130±10.0
Difference	+4	+16	+72	+78	+22	+66	+43±11.0

[a] No manurial effect on this crop.

[1] The probable error of the mean as used in these tables is obtained by Merriman's formula 30, which is stated as follows: $rop=\frac{0.8453\Sigma v}{n\sqrt{n-1}}$. In other words, the probable error is obtained by multiplying the sum of the departures from the mean by the quotient of $n\sqrt{n-1}$ into 0.8453, where n equals the number of yields involved (MERRIMAN, Mansfield. METHOD OF LEAST SQUARES. ed. 8. 1913).

TABLE I.—*Effect of manure on the yields of Irish potatoes at the Scottsbluff, Nebr., Belle Fourche, S. Dak., and Huntley, Mont., field stations, 1912 to 1917*—Continued

HUNTLEY

Rotation No.	1912	1913	1914	1915	1916	1917	Mean.
20 (no manure)	274	200	179	350	228	241	245±16.8
21 (manure)	270	226	176	386	294	277	271±18.0
Difference	−4	+26	−3	+36	+66	+36	+26± 7.5
24 (no manure)	263	316	171	273	236	229	248±13.6
25 (manure)	413	362	201	374	324	292	328±21.0
Difference	+150	+46	+30	+101	+88	+63	+80±12.6
30 (no manure)	208	188	156	228	224	175	196± 8.9
31 (manure)	[a] 199	160	140	273	212	91	179±18.5
Difference	−9	−28	−16	+45	−12	−84	−17± 9.7

[a] No manurial effect on this crop.

The results given in Table I, which refer to the total yield of potatoes, show that in 8 of the 9 cases the application of manure was followed by increased yields, and it should be remembered that these crops were produced on new land, where good yields are obtained even without manuring.

Each year since 1913 it has been the practice in connection with these experiments to sort the potatoes in the field at the time of digging. This sorting has been done by means of a wire screen with 2-inch meshes. The smaller potatoes that pass through this screen are classed as unmarketable, while those passing over the screen are classed as marketable. It has been observed at Scottsbluff and at Belle Fourche not only that the manured crops have been larger than those not manured, but also that the proportion of marketable potatoes has been larger on the manured land. This has not so far proved to be the case at Huntley. The yields of marketable potatoes at the three stations for the three pairs of rotations for five years are given in Table II, together with the annual differences and the mean yields and the mean of the annual differences.

TABLE II.—*Effect of manure on the yields of marketable Irish potatoes at the Scottsbluff, Nebr., Belle Fourche, S. Dak., and Huntley, Mont., field stations, 1913 to 1917, inclusive*

[The yields, differences, and means are expressed in bushels per acre]

SCOTTSBLUFF

Rotation No.	1913	1914	1915	1916	1917	Mean.
20 (no manure)	243	74	47	119	98	116±21.9
21 (manure)	208	156	101	129	153	149±11.7
Difference	−35	+82	+54	+ 10	+55	+33±15.5
24 (no manure)	101	75	68	185	110	108±13.4
25 (manure)	251	190	102	192	164	180±15.8
Difference	+150	+115	+34	+ 7	+54	+72±20.5
30 (no manure)	194	143	101	197	147	156±13.2
31 (manure)	237	168	135	217	184	188±13.1
Difference	+43	+25	+34	+20	+37	+32± 3.1

BELLE FOURCHE

20 (no manure)	87	66	93	86	97	86± 3.3
21 (manure)	104	109	175	117	163	134±12.0
Difference	+17	+43	+82	+31	+66	+48± 8.9
24 (no manure)	89	106	102	112	96	101± 2.9
25 (manure)	75	89	155	96	117	106±10.0
Difference	−14	−17	+53	−16	+21	+5±10.7
30 (no manure)	37	54	48	120	107	73±14.5
31 (manure)	76	120	127	143	182	129±11.2
Difference	+39	+66	+79	+23	+75	+56± 8.6

HUNTLEY

20 (no manure)	192	152	336	201	226	221±19.2
21 (manure)	212	139	359	262	260	246±24.0
Difference	+20	−13	+23	+61	+34	+25±7.6
24 (no manure)	307	149	259	212	213	228±18.8
25 (manure)	347	181	363	298	278	293±21.6
Difference	+40	+32	+104	+86	+65	+65±10.0
30 (no manure)	179	131	212	206	159	177±11.0
31 (manure)	150	115	259	182	83	158±21.2
Difference	−29	−16	+47	−24	−76	−19±11.9

The facts set forth in Tables I and II may be summarized separately for each of the three stations for the five years 1913 to 1917, as follows:

At Scottsbluff the yield of potatoes, large and small, for the 30 plot-years, has averaged 208 bushels per acre, with a mean annual difference in favor of the manuring of 40 ± 7. The yield of marketable potatoes from the same plots has averaged 150 bushels per acre, with a mean annual difference in favor of the manuring of 46 ± 7. The percentage of the total yield classed as marketable for the 30 plots is 72, while the mean annual difference in percentage marketable in favor of the manuring is 8 ± 1.5.

At Belle Fourche the total yield of potatoes for the 30 plot-years has averaged 131 bushels per acre, while the mean annual difference in favor of manuring is 34 ± 6.4. The yield of marketable potatoes from the same plots has averaged 105 bushels per acre, while the mean annual difference in favor of the manuring is 36 ± 6.3. The percentage of the total yield classed as marketable for the 30 plots is 80, while the mean annual difference in percentage marketable in favor of the manuring is 7 ± 1.9.

At Huntley the total yield of potatoes for the 30 plot-years has averaged 239 bushels per acre, while the mean annual difference in favor of manuring is 26 ± 8.3. The yield of marketable potatoes from the same plots has averaged 221 bushels per acre, while the mean annual difference in favor of the manuring is 24 ± 8.5. The percentage of the total yield classed as marketable for the 30 plots is 92, while the mean annual difference has been negligible, being in favor of the manured plots by less than 1 per cent.

SUGAR BEETS

The effect of manure on the yields of sugar beets is shown in Table III, which gives the annual yields in tons per acre for the manured and unmanured plots and the annual differences in yield between the pair of plots that have had similar treatment except for the manuring. The table also shows the mean annual yield of the crop for each rotation for six years and the mean of the annual differences with the probable errors of these means obtained as indicated above in the discussion of Table I.

In the 12 comparisons shown in Table III the mean yields for the 6-year period all show increases as a result of manuring. Some of these increases are too small to be considered significant, but in 9 cases of the 12 the mean of the annual differences exceeds its probable error sufficiently to be regarded as significant. Eliminating the 3 cases of rotation 21 in 1912, in which the manure had not been applied, there remain 69 annual comparisons. Of these annual comparisons 62 show increased yields following the application of manure and only 7 show decreased yields.

In rotation 21 the manure is applied for the potato crop, which precedes the sugar beets, so that the later crop receives only the second-year effect of the manure. In rotations 21 and 23 manure has been applied to the same plot three times during the 6-year period covered by the table.

In rotation 31 manure has been applied to the same plot twice during the period, while in rotation 61 only one cycle has been completed, and no residual effect of previous applications of manure is to be observed.

TABLE III.—*Effect of manure on the yield of sugar beets at the Scottsbluff, Nebr., Belle Fourche, S. Dak., and Huntley, Mont., field stations, 1912 to 1917, inclusive*

[The yields, differences, and means are expressed in tons per acre]

SCOTTSBLUFF

Rotation No.	1912	1913	1914	1915	1916	1917	Mean.
20 (not manured)	15.3	19.6	14.5	10.6	5.8	13.0	13.0±1.3
21 (manured)	[a]13.5	20.8	17.2	13.1	6.4	17.0	14.6±1.4
Difference	−1.8	+1.2	+2.7	+2.5	+ .6	+4.0	+1.6± .58
22 (Not manured)	13.9	18.3	14.2	12.0	7.3	11.1	12.8±1.0
23 (manured)	19.4	21.4	19.9	15.9	9.4	18.6	17.4±1.2
Difference	+5.5	+3.1	+5.7	+3.9	+2.1	+7.5	+4.6± .6
30 (not manured)	17.6	17.0	13.9	10.2	9.8	9.1	12.9±1.2
31 (manured)	21.4	23.8	17.4	15.6	17.1	16.0	18.5±1.0
Difference	+3.8	+6.8	+3.5	+5.4	+7.3	+6.9	+5.6± .52
60 (not manured)	17.3	17.2	14.8	12.5	7.3	15.3	14.1±1.0
61 (manured)	22.4	23.4	18.5	15.3	11.9	19.5	18.5±1.2
Difference	+5.1	+6.2	+3.7	+2.8	+4.6	+4.2	4.4± .33

BELLE FOURCHE

20 (not manured)	7.0	10.7	11.3	12.2	9.7	15.3	11.0±0.7
21 (manured)	[a]7.0	8.8	14.6	16.0	12.1	18.6	12.8±1.3
Difference	.0	−1.9	+3.3	+3.8	+2.4	+3.3	+1.8± .7
22 (not manured)	7.6	7.6	12.3	9.1	8.1	17.7	10.4±1.1
23 (manured)	5.7	8.3	10.6	11.8	9.6	20.9	11.1±1.3
Difference	−1.9	+ .7	−1.7	+2.7	+1.5	+3.2	+ .7± .65
30 (not manured)	7.4	9.1	10.7	7.3	4.6	7.4	7.7±0.6
31 (manured)	8.5	9.3	14.2	13.2	8.2	15.3	11.4±1.1
Difference	+1.1	+ .2	+3.5	+5.9	+3.6	+7.9	+3.7± .8
60 (not manured)	8.5	8.0	11.2	9.4	7.2	12.7	9.5± .6
61 (manured)	7.1	6.1	12.7	10.9	10.4	15.7	10.5±1.0
Difference	−1.4	−1.9	+1.5	+1.5	+3.2	+3.0	+1.0± .56

[a] No manurial effect on this crop.

TABLE III.—*Effect of manure on the yield of sugar beets at the Scottsbluff, Nebr., Belle Fourche, S. Dak., and Huntley, Mont., field stations, 1912 to 1917, inclusive*—Con.

HUNTLEY

Rotation No.	1912	1913	1914	1915	1916	1917	Mean.
20 (not manured)	12.3	12.6	15.1	8.3	15.3	9.6	12.2±0.8
21 (manured)	[a]12.6	13.3	14.4	12.8	17.9	12.1	13.8± .6
Difference	+ .3	+ .7	− .7	+4.5	+2.6	+2.5	+1.6± .59
22 (not manured)	11.7	15.2	10.4	6.8	11.1	11.3	11.1± .6
23 (manured)	14.4	13.4	12.6	10.7	12.0	12.5	12.6± .3
Difference	+2.7	−1.8	+2.2	+3.9	+.9	+1.2	+1.5± .54
30 (not manured)	7.3	11.4	6.6	4.8	6.7	6.7	7.2± .5
31 (manured)	8.3	15.3	12.8	9.1	10.0	9.6	10.8± .8
Difference	+1.0	+3.9	+6.2	+4.3	+3.3	+2.9	+3.6± .45
60 (not manured)	9.7	11.6	9.3	12.9	7.4	8.3	9.9± .6
61 (manured)	12.2	15.8	13.6	8.7	15.6	13.0	13.2± .7
Difference	+2.5	+4.2	+4.3	−4.2	+8.2	+4.7	+3.3±1.03

[a] No manurial effect on this crop.

In addition to recording the yield of beets from the rotation plots as shown in Table III, it has been customary also to determine the average size of the beets on each plot by counting and weighing the product of several representative rows, to record the percentage of sugar in the beets as reported by the local sugar factory upon samples from each plot, and to determine the proportion of the weight of the tops to the combined weight of beets and tops. This last determination has a bearing not only as showing the vigor of growth of the sugar beets, but also as indicating the quantity of feed left as a by-product of the beet crop.

The facts regarding the effect of manure on increasing the yield of beets as shown in Table III, and also as to the effect on size of beets, percentage of sugar in the beets, and percentage of tops, are summarized for each of the three stations as follows:

At Scottsbluff the yield of beets from the 46 plot-years, omitting rotations 20 and 21 in 1912, averaged 15.3 tons per acre, while the mean annual difference in favor of the manuring is 4.3±0.28. The size of beets from the same plots averaged 1.55 pounds, with a mean annual difference in favor of the manuring of 0.32±0.044. The percentage of sugar in the beets was not determined in 1912, so that the results of only 40 plot-years are available. This averaged 16.5 per cent, with a mean annual difference in favor of the unmanured plots of 0.12±0.20, which is not significant. The percentage of the weight of top to the

combined weight of beets and tops for 46 plot-years is 26 per cent, with a mean annual difference in favor of the manuring of 2.7 ± 0.6.

At Belle Fourche the yield of beets for 46 plot-years averaged 10.7 tons per acre, with a mean annual difference in favor of the manuring of 1.9 ± 0.36. The size of beets from the same plots averaged 0.84 pound, with a mean annual difference in favor of the manuring of 0.08 ± 0.035. The percentage of sugar in the beets, omitting 1912, averaged for 40 plot-years 19.6 per cent, with the mean annual difference in favor of the manured plots of 0.3 ± 0.22. The record of the percentage of tops is incomplete.

At Huntley the yield of beets for 46 plot-years averaged 11.3 tons per acre, with the mean annual difference in favor of the manuring of 2.6 ± 0.33. The notes as to size of beets, percentage of sugar, and percentage of tops were not taken for 1912. The size of beets for 40 plot-years averaged 0.92 pound, with the mean annual difference in favor of manuring of 0.21 ± 0.034. The percentage of sugar in the beets averaged 16.8 per cent, with a mean annual difference in favor of the manuring of 0.16 ± 0.17, which is negligible.

The proportion of the weight of tops to the combined weight of beets and tops averaged 32 per cent, with a mean annual difference in favor of the manuring of 3.2 ± 1.12.

SUMMARY

The effect of manure on the yields of Irish potatoes and sugar beets under irrigation has been tested for six years in seven rotations at each of three different stations in the northern Great Plains. Comparison is made between the yields of these crops when grown in rotations without manure and when grown in the same sequence in other rotations in which manure is applied at the rate of 12 tons per acre once during the cycle of the rotation.

At Scottsbluff, Nebr., the effect of the manure has been to increase the yield of potatoes about 40 bushels per acre, to increase the proportion of marketable potatoes about 8 per cent, and to increase the yield of sugar beets 4.3 tons per acre without materially affecting the sugar content of the beets.

At Belle Fourche, S. Dak., the effect of the manure has been to increase the yield of potatoes about 34 bushels per acre, to increase the proportion of marketable potatoes about 7 per cent, and to increase the yield of sugar beets 1.9 tons per acre without materially affecting the sugar content of the beets.

At Huntley, Mont., the effect of the manure has been to increase the yield of potatoes about 26 bushels per acre without influencing materially the proportion of marketable potatoes, and to increase the yield

of sugar beets 2.6 tons per acre without materially affecting the sugar content of the beets.

In five of the seven rotations considered the increased yields were from the crop immediately following the application of the manure. In the other two rotations the yields were from crops produced the second season after the manure was applied. The increases in yield shown in these two cases, as well as the effects observed with other crops grown in these rotations, show that the benefit of the manure is appreciable for two years or more after it was applied.

RELATION OF INORGANIC SOIL COLLOIDS TO PLOWSOLE IN CITRUS GROVES IN SOUTHERN CALIFORNIA

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The term "plowsole" as here used refers to the hard layer of soil artificially formed in Citrus groves in southern California when the soil is intensively cultivated during the irrigation season. Its upper limit is the lower limit of the soil mulch established by cultivation, and it has been found to vary in thickness from a few inches to 2 feet, though usually it does not extend below the first 18 inches of the soil. The hardness of the plowsole varies from a perceptible crust to a layer difficult to penetrate with a shovel. Immediately after irrigation it is usually soft, and is then easily broken; as the soil dries, the plowsole layer hardens again.

If soil from groves, or from the surrounding native desert areas, is put into pots in the laboratory, irrigated, and the surface layer stirred to form a soil mulch, a hard crust will be formed under the mulch by the time all the soil is dry. The soil just below the crust usually retains a crumbly structure, and does not harden. This plowsole occurs as the result of a single irrigation followed by a few surface cultivations. It thus appears that the packing caused by teams and implements is not necessary for its formation.

Plowsole has been found to occur in all soil types investigated in the Riverside, Redlands, and Corona areas. Coarse granitic soils, as well as the heavier clay loam soils, are subject to plowsole formation. It has not, however, been observed to form under the trees where the soil has not been cultivated, especially when an organic mulch has been maintained; nor has it been found in basins mulched with organic matter. When the organic mulch is not maintained, the immediate surface soil crusts; but the crust is formed simply by the drying out of the surface soil.

In many groves the fibrous Citrus roots do not readily penetrate the plowsole, though roots are often found in the hard layer. It appears that in such cases of penetration the roots are formed during the rainy season when the plowsole is soft and maintained in a moist condition. Between irrigations in the summer time the upper part of the plowsole itself dries out below the moisture content necessary to maintain root growth.

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In other groves it was found that the fibrous roots grew freely into the uncultivated surface soil under the trees, but not at all into the plowsole layer formed in the area frequently cultivated.

In addition to checking the root growth and limiting the soil layer available for the root development of the shallow-rooted Citrus trees, the plowsole seriously checks the penetration of the irrigation water.

The condition may be temporarily relieved by breaking up the plowsole layer with a subsoiler, but the plowsole forms again when cultivation is resumed. The best remedy against the formation of plowsole seems to be a surface organic mulch, with no cultivation during the irrigation season beyond that necessary to keep weeds under control.

The present report deals with some of the properties of plowsole as found in citrus groves or formed artificially in the laboratory. The work was done in Riverside, Cal., on soil samples from that area.

COMPOSITION OF WATER EXTRACT OF PLOWSOLE

Soil samples were collected from eight orange groves in which plowsole occurred. Samples at three different depths were collected, including (1) the soil mulch, usually 3 to 5 inches deep; (2) the plowsole crust; (3) the subsoil under the plowsole.

Each sample was rolled, passed throgh a 2-mm. sieve, extracted with distilled water in the ratio of 1 part of soil to 2 parts of water, and the extract analyzed. The water extracts of the soil samples from four of the groves were filtered through Chamberland porous filter tubes, and the water extracts of the samples from the other four groves were filtered through filter paper.

Table I shows the average amounts, in parts per million on the basis of dry soil, of iron, calcium, magnesium, and silica found in the respective soil layers. Each figure is the average in samples from four groves.

The elements determined are those usually considered to have a cementing action in soil, and with one exception there was evidently no accumulation of these in the plowsole. Mineral carbonates were present in the soils included in these analyses only in traces or very small amounts.

The comparatively large amount of iron in the water extracts of the plowsole filtered through filter paper is due to one exceptionally high determination—viz, 9 p. p. m. The average amount of iron in this layer in the three other groves was 1.07 p. p. m., and this amount evidently more nearly represents the true condition in respect to iron. In the large number of iron determinations made in water extracts of soils in the area considered, the highest amount found was 2.7 p. p. m., excepting the one mentioned above. The average amounts of iron in eight other water extracts of soil mulch, plowsole, and subsoil were, respectively, 0.88, 0.58, 0.87 p. p. m. on the dry soil. It would therefore seem that 1.07 p. p. m. of iron in the plowsole is a more reliable figure than 3.05 parts, as shown in Table I, when the water extracts were filtered

through filter paper. Unfortunately these soil samples were discarded before this apparent error was detected, the analytical work having been done in May, 1916, and the results not having been carefully tabulated and compared until April, 1917, owing to other work.

TABLE I.—*Analysis of water extracts of soil mulch, plowsole, and subsoil from orange groves. Average of four groves*

[Results reduced to basis of dry soil and expressed as p. p. m.]

Soil layer.	Through Chamberland filters.				Through filter paper.			
	Iron.	Calcium.	Magnesium.	Silica.	Iron.	Calcium.	Magnesium.	Silica.
Mulch	0.61	117	17	13	0.94	70	17	30
Plowsole	.28	23	4	9	[a] 3.05	35	7	27
Subsoil	.31	41	4	9	1.40	29	10	23

[a] Leaving out one exceptionally high determination, the average of the other three groves is 1.07 p. p. m. of iron (see text).

Flowerpots were filled with soil from some of these groves, placed in the laboratory, irrigated several times, and kept mulched. A number of inorganic salts, carbonates, chlorids, nitrates, and phosphates, were mixed with some of the soils. Plowsole layers formed readily under the soil mulch.

After about one month the soil mulch, plowsole, and subsoil were separately extracted with distilled water and analyzed. There was no accumulation of the water-soluble cementing elements in the plowsole layers in either the treated or untreated soils.

The plowsole layers in the differently treated soils varied greatly in hardness. The hardest layers occurred in the soils treated with sodium chlorid, sodium nitrate, sodium phosphate, magnesium sulphate, and sodium carbonate. The softest layers were formed in the soils mixed with calcium carbonate, calcium sulphate, and alfalfa.

Soils from the four orange groves, the soil analysis of which were presented in Table I, were extracted with 1 per cent hydrochloric acid until calcium was absent, washed with distilled water, and then extracted with 4 per cent ammonium hydrate. The ratio of soil to ammonium hydrate was 100 gm. to 500 cc. The "humus" extracts were evaporated to dryness and ignited just enough to drive off the organic matter. There were no facilities on hand at the time for fusion, so the residues were digested with nitrohydrochloric acid on the water bath until no perceptible residue remained.

Partial analyses of these dissolved residues are given in Table II. Each figure represents the average percentage obtained on the analysis of extracts of soils from eight groves.

TABLE II.—*Analysis of humus extracts of soil mulch, plowsole, and subsoil from orange groves. Average percentage of eight groves*

Soil layer.	Percentage, on basis of dry soil, in ammonia extract.			
	Silicon dioxid.	Ferric oxid.	Aluminium oxid.	Phosphorus pentoxid.
Mulch	0.032	0.006	0.015	0.013
Plowsole	.027	.008	.011	.012
Subsoil	.024	.007	.011	.011

There is no evident accumulation of these elements in the plowsole that would account for its formation.

Another set of ammonium extracts was made from samples of the same soils. The colloidal material was precipitated with a little nitric acid, and filtered out. The filtrate was next treated with a little ammonium carbonate, and again filtered. The two precipitates were taken up with a little ammonium hydrate, evaporated to dryness, and ignited just enough to burn off the organic matter. The ignited material was digested as before with nitrohydrochloric acid on the water bath until no perceptible residue remained, and the solution was analyzed. Table III gives the results of the partial analyses made, each figure being the average percentage found in soils from eight different groves.

TABLE III.—*Analysis of inorganic colloids in humus extracts of soil mulch, plowsole, and subsoil from orange groves. Average percentage in soils from eight groves*

Soil layer.	Percentage on basis of dry soil.				
	Silicon dioxid.	Ferric oxid.	Aluminium oxid.	Phosphorus pentoxid.	Silica, iron, and aluminium.
Mulch	0.016	0.005	0.016	0.004	0.037
Plowsole	.030	.008	.025	.004	.063
Subsoil	.015	.006	.018	.003	.039

There is thus an accumulation of colloidal iron, aluminium, and silica in the plowsole layer. The detailed analyses showed this to be consistently the case for each grove.

The analyses given in Tables II and III were made on ammonia extracts of the same composite samples, but each table represents a separate extraction on fresh samples. Hence, the ratio of the colloidal elements to the total amounts of these same elements determined in the ammonia extracts can not be definitely stated from the data obtained. A comparison of Tables II and III, however, would indicate that only about one-third of phosphoric acid was present in the colloidal form in the

ammonia extract, and that practically all the iron was present in that form. It would also appear that practically all the silica and aluminium in the plowsole layer were colloidal.

Fraps (*4*)[1] used ammonium carbonate to precipitate the inorganic soil colloids from ammoniacal solutions. It was found in this work that a little more inorganic material was obtained by precipitating the extract successively with nitric acid and ammonium carbonate. The acid carried down practically all the organic matter as well, as judged by color.

The following experiment shows the results obtained by precipitating the colloids from ammonia extracts of a soil, with nitric acid and ammonium carbonate as precipitants. Two 150-cc. portions of a composite ammonia humus solution were treated separately with nitric acid and ammonium carbonate. The filtrate from the acid-treated extract was then treated with ammonium carbonate, and the filtrate from the carbonate-treated extract was treated with nitric acid. Each separate precipitate was ignited and weighed. The results obtained are given in Table IV.

TABLE IV.—*Comparison of efficiency of nitric acid and ammonium carbonate in precipitating inorganic colloids from ammonia extracts of soil*

First precipitant.	Weight of ignited precipitate.	Second precipitant.	Weight of ignited precipitate.
	Gm.		*Gm.*
Ammonium carbonate	0.0200	Nitric acid	0.0020
Nitric acid	.0202	Ammonium carbonate	.0049

It appears that these two precipitants were about equally effective in precipitating the inorganic colloids from humus solutions. When the two were used successively, an appreciable increase in inorganic material was obtained. The results of the first precipitations would indicate that the flocculated organic matter caused by addition of nitric acid did not carry down or occlude noncolloidal mineral substances.

Samples of soil mulch, plowsole, and subsoil from four other orange groves not included in the discussion so far, were extracted with hydrochloric acid till calcium was absent, washed, and extracted with ammonium hydrate. The inorganic colloids were precipitated as just described, ignited, and weighed. The average percentage of total inorganic soil colloids in the humus extracts of the separate soil tamples from the four groves was as follows: Soil mulch, 0.0642; plowsole, 0.0912; subsoil, 0.073.

These results agree with those given in Table III, in showing that an accumulation of ammonia-soluble inorganic soil colloids occurs in the plowsole layers.

[1] Reference is made by number (italic) to "Literature cited," p. 518–519.

The organic and inorganic colloidal material in these humus extracts evidently moves with the electric current, as the following experiment shows:

Two tubes were connected together with rubber tubing into the form of a U-tube, and filled with humus extract. Electrodes from a battery of six new dry cells connected in series were inserted into the tops of the U-tube touching the extract, and left undisturbed for seven days. The liquid in the two tubes was then separated by closing the rubber-tube connection. A colorimetric humus reading was made of the liquid in each tube. The inorganic colloids were precipitated with nitric acid and ammonium carbonate, ignited and weighed. Table V gives the results obtained.

TABLE V.—*Inorganic colloids and humus in humus extracts of soil in positive and negative sides of the U-tube*

Electric charge in U-tube.	Percentage of inorganic colloids in solution.	Percentage of humus in solution.
Positive	0.0380	0.0625
Negative	.0230	.0500

In the preceding discussion it has been shown that there is evidently no accumulation of water-soluble iron, calcium, magnesium, or silica in the plowsole layers that could account for its formation; that the total ammonia-soluble silica, iron, aluminium, or phosphoric acid could not account for the formation of the plowsole; and that there was accumulation of ammonia-soluble colloidal silica, iron, and aluminium, in the plowsole, but not of colloidal phosphoric acid.

The last-mentioned fact indicated the desirability of making a direct study of the inorganic colloidal material in the soil.[1]

The term "colloid suspension" will be used frequently in what follows, and for the purpose of clearness it will be used to mean simply the inorganic material of a soil remaining in suspension at the end of 24 hours after the soil has been treated in a certain manner.

The following conventional method was adopted for determining the percentage of colloid suspension in the soils. Five gms. of soil were placed in a 250-cc. Erlenmeyer flask, 50 cc. of distilled water was added, the flask covered with a watch glass and placed on the water bath. The contents were shaken periodically, and after 1½ hours the suspended material was decanted into a test tube about 3 cm. in diameter and 25

[1] A discussion of the general properties of colloids will not be entered into here. Whether colloids are classified according to size, chemical constitution, or method of preparation will have but little bearing on the nature of the present work. Excellent discussions on colloids are given by Ostwald (OSTWALD, Wolfgang. HANDBOOK OF COLLOID-CHEMISTRY, . . . Trans. from 3d German ed. 278 p., 60 fig. Philadelphia); and by Burton (BURTON, E. F. PHYSICAL PROPERTIES OF COLLOIDAL SOLUTIONS. 200 p., 18 fig. London, New York), not to mention many other earlier workers in the field.

cm. high. Another portion of 50 cc. of distilled water was added to the soil remaining in the flask, and the former treatment repeated. The same treatment was repeated a third time, and on the third decantation all the soil was washed into the test tube. The tube finally contained about 180 cc. and was left undisturbed for 24 hours. The suspended material was then carefully siphoned off and precipitated with neutral ammonium sulphate. The precipitation was hastened by warming. The precipitate was filtered, ignited, and weighed as the percentage of "colloid suspension."

Duplicate determinations were made, and the departure varied from nothing to a maximum of about 5.5 per cent. When the departure did not exceed 3.5 per cent the results were used. When the error was greater, duplicate determinations were again made.

It was found to be more difficult to obtain two uniform duplicate 5-gm. soil samples than to make good duplicate determinations on a very uniform sample of soil "colloid" used for experimental purposes. To insure better duplicate sampling for colloid determinations, the soil as taken from the groves was rolled and sifted through a 2-mm. sieve.

It is realized that this method of measuring the colloidal content of soils does not show the absolute amount of colloid in a soil. The colloidal content by this method is necessarily affected by tle amount and character of the electrolytes going into solution. The purpose of the measurements was not to get a measurement of the absolute colloidal content, but to study the relative colloidal content in the three layers of soil in plowsole groves. Ashley (*1*) measured the colloid matter in clay by the absorption of dyes in the study of the plasticity of clay. His work has a special bearing on the ceramic industries. This method does not appear applicable to the present work, since it is probable that not only the colloid matter remaining in suspension after 24 hours, but the colloidal matter not remaining in suspension as well would absorb dyes; and so in all likelihood would the organic matter in the soil, which was found to bear no relation to the percentage of inorganic colloid suspension in the soil. The purpose of the present study was to obtain a measurement of the colloid matter that was free to move with the soil moisture, as it seemed to do.

That the method of determining the colloid suspension as above described is entirely conventional is shown by the following experiment. Five-gm. soil samples from a composite sample were put into Erlenmeyer flasks, distilled water was added, and the flasks were placed on the bath. The total amount of water used and the total time of the sample on the bath were the same for each determination. In each case, also, the suspended matter as transferred to the test tubes stood 24 hours before being siphoned off. The variable factor was the number of decantations and consequently the amount of water used for each. Table VI gives the results of the determinations and the condition of each test.

TABLE VI.—*Inorganic colloid suspension in soil as influenced by successive decantations with small amounts of distilled water and by single decantation with larger amounts of water, total time on water bath being the same in all cases*

Number of decantations.	Quantity of water with each decantation.	Time on water bath each decantation.	Percentage of colloid on soil.
	Cc.	*Hours.*	
3	60	2.5	0.476
2	85	3.75	.418
1	175	7.5	.338
1	[a] 175	7.5	.847

[a] 3 cc. of ammonium hydrate added.

Soil mulch, plowsole, and subsoil samples taken in groves having plowsole, were rolled (not crushed), sifted through a 2-mm. sieve, and subjected to colloid-suspension determinations. The average percentage of the inorganic colloid suspension in soil samples from seven different groves was as follows: Mulch, 0.879; plowsole, 1.334; subsoil, 1.215. In all groves but one the colloid content was greater in the plowsole than in either the soil mulch or subsoil. The grove which proved the exception was sampled a second time and subjected to colloid-suspension determinations, which gave results similar to the first determinations.

There is thus an accumulation of inorganic colloid matter in the plowsole layer, especially as compared with the soil-mulch layer.

From laboratory experiments it would appear that this colloid matter migrates with the soil moisture much as do soluble salts, though probably much more sluggishly. Soils from groves were put in flowerpots in the laboratory and irrigated, the soil being allowed to dry out before each irrigation and before the colloid determinations were made. In every instance after one or more irrigations the surface layer of soil was found to contain a greater percentage of inorganic colloid suspension than the subsurface soil. The increase in colloid matter in the surface layer varied from 11 to 70 per cent.

The inorganic colloid condition of the soil is largely governed by the composition of the soluble salts present. The sulphates of sodium, ammonium, and calcium tend to precipitate the colloids, while the nitrates, carbonates, and bicarbonates have the reverse effect.

It is not uncommon to find considerable accumulation of "alkali" salts on the irrigation furrow slopes in Citrus groves after irrigation. Breazeale (*2*) has analyzed some of these and found the predominant salts to be sulphates and nitrates, the latter being present in the greater quantity. McBeth (*6*) has shown that nitrates are at times present in large amounts on the slopes of the irrigation furrows. The latter's work shows also the relation of nitrate accumulation to the rainfall, which is of importance in the present discussion. Finally the irrigation

waters used in the area here under consideration carry more bicarbonates than sulphates (*3*).

As a whole the deflocculating salts predominate over the flocculating salts in the cases examined by the Office of Biophysical Investigations in Citrus groves in southern California. Hence, so far as the readily soluble soil salts are concerned, the tendency would be for the inorganic colloid matter to be carried down into the soil by irrigation water and rains. As a surface soil mulch is established in most cases as soon as possible, the colloid matter would naturally tend to remain below this layer.

After the winter rains it was found that the amount of electrolytes in the plowsole layer exceeded that in the soil surface by about 60 per cent, as determined by conductivity measurements. The accumulation of soluble salts in the plowsole layer would probably tend to precipitate the colloids, and the denser physical structure of this layer would act as a filter.

No relation could be established between the percentage of colloid suspension and the percentage of humus or organic carbon in the soil. The addition of organic matter to soil, however, was found to influence the inorganic colloid state in the soil. Various organic substances were added to soils from orange groves, and the treated soils put into flowerpots in the laboratory. These were irrigated intermittently for a period of over one year. The soils were then taken out, rolled, sifted through a 2-mm. sieve, and subjected to inorganic colloid-suspension determinations. Table VII gives the results obtained.

TABLE VII.—*Effect of the addition of organic matter to soils on the amount of inorganic colloid suspension. Organic matter in contact with the soils for more than a year*

Organic treatment.	Percentage of inorganic colloid suspension.				
	Sandy loam.		Clay loam.		
	Manure (soil C).	Alfalfa (soil A).	Manure (soil D).	Alfalfa (soil F).	Barley (soil H).
Untreated	0.422	0.511	0.764	0.778	0.852
1 per cent of organic matter	.314	.....	.855	.965	.707
3 per cent of organic matter	.229	.372	.755	.787	.675

In most cases the addition of 3 per cent of organic matter decreased the amount of inorganic colloid matter, while 1 per cent had less effect. Apparently the state of the colloid matter was more influenced by organic treatment in the sandy-loam soil than in the clay-loam soil.

It is possible that the effect of the organic matter on the amount of colloid suspension in the above experiment is due in part to the indirect effect of the change in content of soluble salts. It has been found that the addition of organic matter to soils very appreciably affects the solu-

bility of the soil minerals (5). The nature of the compounds thus produced has not been investigated, nor has the effect of the by-products of the decomposition on the inorganic colloid matter of the soil.

The addition of 1 per cent of organic matter represents approximately 10 tons dry matter per acre 6 inches. This is probably more than is usually applied per acre at any one time in commercial citriculture in southern California. The improvement in the physical condition of the soil by the addition of organic matter in the customary amounts would thus hardly seem to be due to any marked changes in the state of the inorganic colloid matter of the soil but rather to the mechanical separation of the soil aggregates.

Soil from the same groves represented in Table VII was treated with certain organic and inorganic substances, put into pots in the laboratory The soils were irrigated intermittently for over a year. The soils were then rolled, passed through a 2-mm. sieve, and subjected to inorganic colloid determinations. Table VIII gives the results obtained.

TABLE VIII.—*Effect of the addition of certain organic and inorganic substances to soils on the amount of inorganic colloid suspension. Substances in contact with the soils for more than a year*

Inorganic treatment.	Percentage of inorganic colloid suspension.							
	Clay loam.						Sandy loam, organic treatment (soil C).	
	No organic treatment (soil B).	Organic treatment.						
		Soil D.		Soil F.		Soil H.		
		Control.	3 per cent manure.	Control.	3 per cent alfalfa.	Control.	3 per cent barley.	Control.	3 per cent manure.
Control:									
Untreated	0.931	0.764	.....	0.778	.....	0.852	.....	0.422	.....
Organic matter alone (3 per cent)	.....	.....	0.755	.....	0.787	.....	0.075	.....	0.229
Lime carbonate:									
1 per cent	.540	.....	.707	.....	.614	.....	.584	.....	.214
3 per cent	.517	.....	.579	.....	.611	.....	.497	.....	.202
Sulphur:									
0.5 per cent	.234	.....	.....	.....	.210	.....	.193	.....	.....
0.2 per cent	.....	.....	.520	.....	.....	.....	.....	.....	.200
Sodium nitrate	1.206	.....	.839	.....	.991	.....	.870	.....	.235
Gypsum	.244	.....	.211	.....	.280	.....	.330	.....	.208

All the clay-loam soils are from the same large composite sample, but owing to the impossibility of selecting samples entirely comparable, each soil number should be considered as a separate experiment. It would seem evident, however, that the organic matter tended to counteract the flocculating effect of the lime in the clay-loam soil. The organic

matter also counteracted the deflocculating effect of the nitrate. The barley was more effective in these respects than the other organic substances. Organic matter showed no consistent effect on the flocculating effect of sulphur and gypsum. In the sandy-loam soil it is apparent that the addition of lime to the organic matter had but little effect in decreasing the inorganic colloid matter.

The action of the sulphur in flocculating the colloid matter is probably due to the formation of sulphates, which are good colloid precipitants.

Groves that had been in cultivation many years contained a much greater percentage of colloid suspension than the corresponding native-soil type adjoining.

The composition of the colloid suspension obtained from soils differently treated was partially investigated. The colloid was obtained as previously described, precipitated, ignited, weighed, and fused with anhydrous sodium carbonate in platinum crucibles. The resulting cake was taken up with distilled water and partially analyzed.

The soils used in these determinations had been treated as shown in Table IX, put into flowerpots, and irrigated and cultivated intermittently for over a year before these determinations were made.

TABLE IX.—*Partial analysis of whole soil and of inorganic colloid suspension obtained from same soil receiving certain organic and inorganic treatments. Clay-loam soil from near Riverside, Cal., of granitic origin*

Soil treatment.	Colloid in soil.	Silicon dioxid.	Perric oxid.	Aluminum oxid.	Calcium oxid.	Manganous oxid.	Phosphorus pentoxid.
	Percent.	*Percent.*	*Percent.*	*Percent.*	*Percent.*	*Percent.*	*Percent.*
Untreated	0.682	52.0	12.7	27.1	1.3	1.4	0.24
3 per cent of manure	.643	49.9	12.3	27.2	1.6	1.2	.31
3 per cent of manure + 3 per cent of calcium carbonate	.439	51.4	12.6	27.0	2.7	1.9	.19
3 per cent of manure + .2 per cent of sulphur	.578	50.8	13.7	25.5	2.8	1.5	.64
3 per cent of manure + .2 per cent of sodium nitrate	.737	47.0	11.8	27.8	2.9	1.3	.50
3 per cent of manure + 1.8 per cent of iron sulphate	.375	49.6	12.7	27.9	3.6	2.5	.48
3 per cent of manure + 3 per cent of calcium sulphate	.244	53.8	10.0	26.0	6.2	3.0	.62
Whole soil	.....	61.4	4.7	17.3	3.5	0.6	.18

Table IX shows the results of the partial analysis of the whole soil and of the colloid suspensions. Two evaporations were made in the silica determinations, but the silica residue was not purified with hydrofluoric acid. In this connection it may be stated that the fused cake, when taken up with water, never contained any visible undissolved residue.

The composition of the colloid suspensions from the differently treated soils does not vary enough to indicate any fundamental differences due to the soil treatments, except when gypsum was added. All other

analyses made of colloid suspensions not here included agree in showing lower iron and higher silica, calcium, and manganese in this colloid suspension than in the other suspensions. The variation in the composition of the other colloid suspensions is probably due to analytical errors. The low silica obtained on the colloid from the nitrate-treated soil is probably an analytical error, as other determinations on colloid from similarly treated soil gave about the same percentage of silica as obtained on the other colloids.

Fraps (4) found that the ammonia-soluble inorganic soil colloids varied in composition with the percentage of colloids in the soil. The percentage of silica decreased and the percentage of aluminium increased with increasing percentages of total colloid, in soils of different character and from different localities.

There is a marked difference between the composition of the whole untreated soil and of the inorganic colloid suspensions obtained from it. Aside from the difference in the silica, the differences in the iron and aluminium are evidently significant, especially in the light of determinations made on a soil of different physical and mechanical properties.

A heavy adobe soil from near Whittier, Cal., was treated with different inorganic substances. Determinations of inorganic colloid suspension were made, and the colloid partially analyzed. Table X gives the results obtained.

TABLE X.—*Partial analysis of whole soil, and of inorganic colloid suspensions obtained from the same soil after having received different inorganic treatments. Hanna adobe soil, near Whittier, Cal.*

Soil treatment.	Colloid in soil.	Silicon dioxid.	Ferric oxid.	Aluminium oxid.	Calcium oxid.	Manganous oxid.	Phosphoric pentoxid.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Untreated	0.823	60.7	11.5	22.0	2.3	0.90	0.10
Calcium carbonate 3 per cent	.558	58.8	11.0	21.7	2.4	1.44	.48
Sodium nitrate 0.2 per cent	.803	59.1	11.9	20.7	1.8	1.57	.38
Ammonium sulphate 0.2 per cent	.870	58.2	10.4	20.9	2.0	2.14	.25
Whole soil	....	60.8	3.4	14.2	4.5	.30	.08

The analysis of the inorganic colloid suspensions obtained from the adobe soil under various treatments does not indicate any fundamental differences in the composition due to these treatments.

The percentage of iron and aluminium in the colloids is markedly higher than in the original soil, the same relation as was found in the soil from Riverside (Table IX). The percentage of silica in the original adobe soil is about the same as in the colloid suspensions obtained from it.

A comparison of Table IX with Table X shows that the percentage of iron and aluminium in the colloid suspensions obtained from the Riverside soil is higher than that in the colloid suspensions obtained from the

adobe soil. The Riverside soil is very prone to form plowsole, more so than the Whittier soil. This may be due to the higher content of iron and aluminium in the inorganic colloid suspension.

Stewart (7) suggests that the plasticity of clay is due to the presence of organic aluminium compounds. The chemical association of aluminium with organic substances in the colloid suspensions here studied has not been examined.

SUMMARY

A hard soil layer, here termed "plowsole," usually forms immediately under the soil mulch in cultivated Citrus groves in southern California. It often seriously limits the root system of the shallow-rooted Citrus trees, and seriously interferes with penetration of irrigation water. After being broken up with a subsoiler, it reforms when cultivation is resumed.

It does not form in mulched basins, nor seriously in groves surface mulched with organic matter and seldom cultivated.

Mechanical packing is not necessary for its formation, and it forms in all soil types examined in the area studied.

No greater accumulation of water-soluble iron, calcium, magnesium, or silica was found in the plowsole than in the soil mulch or subsoil.

No greater accumulation of total ammonia-soluble silica, iron, aluminium, or phosphoric acid was found in the plowsole than in the soil mulch or subsoil, after the calcium had been removed with hydrochloric acid.

The humus extract of plowsole contained more colloidal silica, iron, and aluminium than either the soil mulch or subsoil, but no more colloidal phosphoric acid.

Both the organic and inorganic colloidal material in the humus extract moved toward the positive pole in an electric current.

A conventional method for the determination of inorganic "colloid suspension" is given.

Plowsole contained a markedly higher percentage of inorganic colloid suspension than the soil mulch, and usually a higher percentage than the subsoil.

When soils were placed in pots in the laboratory, irrigated, and allowed to dry, the percentage of colloid suspension was found to be appreciably greater in the surface soil layer than in the subsurface layer, indicating that the colloids moved with the capillary soil moisture.

No relation could be observed between the percentage of inoragnic colloid suspension and the percentage of organic carbon or humus in the soil.

Native uncultivated soils contained appreciably less colloid suspension than did similar soils which had been under cultivation for a number of years.

The decomposition of 1 per cent organic matter in soil had no marked effect on the percentage of inorganic colloid suspension; 3 per cent organic matter in some cases decreased the per cent of colloids. Barley decreased the amount of colloid suspension more than did alfalfa or manure.

The addition of ground lime rock appreciably decreased the percentage of inorganic colloid suspension in the soil when no organic matter was added. When organic matter was added, the flocculating effect of lime was appreciably diminished, especially in clay loam soil.

The addition of powdered sulphur and gypsum to soil markedly decreased the colloid content, and organic matter had no appreciable effect in counteracting the flocculating effect of these substances.

The addition of sodium nitrate to soil markedly increased the colloid content, and the addition of organic matter appreciably decreased the deflocculating effect of this compound.

The addition of lime, sulphur, sodium nitrate, iron sulphate, ammonium sulphate, and organic matter to soils did not fundamentally change the composition of the inorganic colloid suspension obtained from the soil.

The addition of gypsum to soil increased the percentage of silica, calcium and manganese, and decreased the percentage of iron in the colloid suspension.

The inorganic colloid suspension contained an appreciably higher percentage of iron, aluminium, and manganese than the untreated soil.

In soils which readily form plowsole the percentage of silica in the colloid suspension was also appreciably higher than in the untreated soil.

The percentage of iron and aluminium in colloid suspensions from soils which readily form hard plowsole was higher than in colloid suspensions from soils which do not form a hard plowsole.

The percentage of iron and aluminium in the colloid suspension from a soil was found to be directly correlated with the readiness with which the soil formed plowsole.

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SEEDLING DISEASES OF CONIFERS

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INTRODUCTION

Because of the high cost of seed and the slow growth and delicate character of seedlings during the first few weeks after germination losses caused by the disease known as damping-off have been an important factor in the propagation of conifers. The fact that a number of damping-off parasites are able to cause practically identical symptoms, and the further fact that certain physical factors sometimes cause injury resembling damping-off, has made a study desirable, both of damping-off and of the other diseases which may attack seedlings of the same age. The present paper will consider all the diseases which the writers have found attacking seedlings up to the age of approximately two months.

DAMPING-OFF

Damping-off is the most serious of the diseases attacking coniferous nursery stock in most regions. There is considerable literature on the disease both in connection with conifers and with truck crops. The most important work in relation to conifers is summarized by Spaulding (*25*).[2] Both etiology and control are, nevertheless, seriously in need of further investigation, because of the complications arising from the multiplicity of hosts and parasites involved and from the soil-inhabiting tendency of several of the parasites. A summary of the available data on control has been recently published (*16*).

TYPES OF DAMPING-OFF IN NURSERIES

The old conception of damping-off seems to have been the death of seedlings as the result of the attack of a parasitic fungus at the soil surface, causing a local constriction of the stem at that point, followed by the fall and wilting of the seedling. It appears that several parasites

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[2] Reference is made by number (italic) to "Literature cited," pp. 556–558.

may attack seedlings in this manner, but that they also very frequently attack the seedlings in other ways, while the cases which most closely agree with the older descriptions of damping-off are often not caused by parasites, and may occur under hot, dry conditions. The term "damping-off" as here used will include all cases of the death and early decay of seedlings less than 2 months old, resulting primarily from fungus invasion. Consideration of the disease as thus limited will therefore involve the discussion both of the work of several different parasites and of several symptomatic types. Many of these types intergrade to such an extent that there is no possibility of giving them separate treatment as distinct diseases. The different types recognized are described in the following paragraphs.

NORMAL DAMPING-OFF

Normal damping-off is caused by *Pythium debaryanum*, *Fusarium moniliforme*, *F. ventricosum*, *Corticium vagum* (the common American Rhizoctonia), and several other fungi. The still succulent seedlings are invaded by the parasites at any point on the root or lower part of the stem, ordinarily a short distance below the ground surface. The parasites spread rapidly, especially through the root tissues, and the seedlings fall over. The fall of the seedlings is not usually due to stoppage of water supply and consequent wilting; it most commonly occurs when the tissues of the hypocotyl just above the soil surface become involved in the decay, and while the rest of the stem is still green and turgid. This type of disease is shown in Plate B, figure 1. It may be fairly well controlled by soil disinfection.

GERMINATION LOSS

This type of damping-off seems in most cases to be caused by species of Pythium and Corticium rather than by Fusarium. The radicles are killed soon after emerging from the seed coats and before the seedlings appear above the ground. Two-thirds of the seedlings are sometimes destroyed in this way in nursery beds. There is no essential difference between this type and the normal type, except that this type occurs earlier in the life of the seedling and is ordinarily not detected. A poor initial stand of seedlings due to this type of damping-off is commonly attributed by the nurseryman not to damping-off but to poor germinative capacity of the seed. This early type of damping-off, like the normal type, is invariably fatal. It can be much more completely prevented by soil disinfection than can the more familiar normal type.

It is often stated by practical men that seedlings from seed with low germination percentage are especially liable to attack by damping-off fungi. This is very likely true within certain limits. However, in a great many cases in which apparently poor germination has been followed by especially heavy damping-off losses, the sequence is to be

attributed not at all to poor seed, but rather to abundant early infection of beds with species of Pythium or Corticium, causing both the apparently poor germination and the high damping-off loss.

LATE DAMPING-OFF

The term "late damping-off" is used for the damping-off as a result of root infections of seedlings several weeks old whose stems have developed strong supporting tissues.

The parasites which cause normal damping-off are also probably responsible for most cases of this type. Like germination loss, it differs from the normal type only by the age of the seedlings concerned. However, the symptoms of late damping-off are very different from those of the normal type; the seedlings remain erect, dry up and turn brown, and in some cases even shed their leaves before the stem finally falls over (Pl. B, fig. 6). This type is very likely to be confused with drouth injury.

The death of seedlings due to root killing by damping-off parasites in rare cases continues throughout the season, and probably even into the second year in cases where development of the host species has been especially slow. This appeared to be the case with Engelmann spruce (*Picea engelmanni*) at a mountain nursery in New Mexico. All such death, up to the rather arbitrary age of two months, is classed by the writers as damping-off, while similar death of still older stock is classed as rootrot. This time limit is justified only by convenience. The damping-off parasites do practically all of their damage at most nurseries before the seedlings are 2 months old. Their work up to that time is fairly easy to detect and distinguish from disease due to other causes, because of the localization of most of the affected plants on the margins of the old damping-off patches of normal type. Furthermore, the soil treatments which control the normal type of damping-off are also useful, at some places at least, in lessening damage from the late type of disease. It is therefore easiest to consider all the trouble during the first two months as damping-off, without trying to mark any of the intermediate stages as distinct diseases.

The death of stock over 2 months old, on the other hand, will require both different investigative methods and, very likely, different types of control measures. Although sometimes caused by damping-off organisms, there seems to be no practical advantage in considering it at the same time as damping-off.

The impression prevalent in some places that damping-off organisms always infect at the soil surface is shown to be especially incorrect in the case of this late type of damping-off. In one case the root of a young seedling of western yellow pine (*Pinus ponderosa*), growing rapidly in sandy soil at the outer margin of a damping-off patch, was found to have been attacked at a point 11 inches below the soil surface. Not all

the seedlings affected by late damping-off are killed by the parasites. Many of them, as in the case of the "root-sick" sugar-beet seedlings described by Edson (7), have only the youngest portions of their roots killed, and are able then to resist further attack, and so recover. Pine seedlings on the margins of damped-off areas can be found at the age of 6 to 7 weeks, with more than half of their root systems decayed, but with the older parts of the root intact and with vigorous laterals starting from the terminal of the healthy portion. That such seedlings are capable of recovery was demonstrated by replanting a number of badly injured specimens of Corsican pine (*Pinus nigra poiretiana*)[1] and observing their growth during the subsequent weeks.

Pines affected with the late type of damping-off show no external evidence of disease until they are practically dead. Infected seedlings which recover do not, like the root-sick sugar beets described by Edson, exhibit noticeably arrested growth and a flabby appearance of the tops.

DAMPING-OFF OF TOPS

A type of damping-off involving parts of the cotyledons or the upper stem, while the lower stem and root remain sound until after the death of the parts above, is fairly common under moist atmospheric conditions, as in greenhouses, although seldom as prevalent in open seed beds as the normal type of damping-off in which the root or the stem at the ground line is first attacked. Infections are believed in the main to be due to Fusarium. Under extremely moist conditions the mycelium may spread directly to the other seedlings whose cotyledons touch the diseased plant. Soil disinfection is, as would be expected, of relatively little value in preventing this disease of the tops. Avoidance of excessive atmospheric humidity appears especially important for control of this type.

A special case of damping-off of tops of seedlings is found in cases in which the tips of all the cotyledons are simultaneously killed. This at first suggests insufficient water supply as the cause, but examination of younger seedlings shows it to be parasitic. Infection occurs in such cases while all the tips are still inclosed in the persistent seed coat. This type of damping-off is thought to be caused most commonly by species of Fusarium, but it has been observed in pots whose soil had been inoculated with *Corticium vagum*, though absent in parallel control pots. It is also possible that *Pythium debaryanum* may be a cause of this type, as there is abundant opportunity for infection to occur before the tips of the cotyledons and the seed coat they carry are released from the soil. Soil disinfection has apparently decreased losses from this type of damping-off.

[1] The nomenclature used in this paper for foreign trees follows BAILEY, L. H. STANDARD CYCLOPEDIA OF HORTICULTURE. New York, 1916; and for native trees follows George B. SUDWORTH in various publications of the Forest Service, United States Department of Agriculture.

Both these types of damping-off of tops commonly kill the affected seedlings, but not always. Stem infections regularly cause death, but lesions originating in the cotyledons are often arrested before they progress far enough to kill the growing point in the center of the whorl of cotyledons.

BLACKTOP

This type of damping-off known as black top is simply a special case of the preceding from which it is distinguished by the dark color of the tissues decayed. It is illustrated in Plate B, figures 2 and 3. Infection takes place at any point on the stem or cotyledons of very young seedlings. Both infection and the extension of the lesion seem to depend on special weather conditions. There is reason to believe that a species of Trichoderma is the direct cause. This type of damping-off is seldom sufficiently prevalent to be of importance. Soil disinfection with acid is apparently ineffective in preventing blacktop damping-off.

DECAY OF DORMANT SEED

It is undoubtedly true that dormant seed are sometimes killed by microorganisms. Under ordinary seed-bed conditions, species of Pythium and Corticium probably kill some coniferous seed before the coats are split, although this has yet to be demonstrated. It is also considered probable that under certain conditions considerable quantities of seed are destroyed by ordinarily saprophytic molds, which possibly reverse the usual action of the damping-off parasites by attacking the cotyledons before the embryonic radicle is invaded. Seed of jack pine (*Pinus banksiana*) shipped moist in a tin container has been found seriously molded with a species of Penicillium fruiting vigorously on the outside of all the affected seeds. It is commonly stated that the seeds of many crop plants rot in the soil during prolonged wet weather. To what extent coniferous seed suffer from microorganisms before beginning to germinate can not be stated without further investigation. Although perhaps not strictly a damping-off problem, it is certainly one that can be considered to advantage in connection with work on damping-off proper.

TYPES OF DAMPING-OFF IN FORESTS

In direct seeding by the seed-spot method in northern Idaho, the writers are advised by Mr. E. C. Rogers, of the Forest Service, that a considerable proportion of the seedlings die from what appears to be damping-off, and that cultural examinations have shown *Rhizoctonia* sp. in the majority of the cases.

In natural reproduction normal damping-off at least in most forests appears to be a much less important factor than in nurseries. Serious damping-off should be expected only where seedlings come up in groups, or where soil corditions especially favor parasites.

In cases of conifers which fail to reproduce well in forest soils containing much humus or covered with litter, the possibilities of parasitic germination loss or of the decay of seed before germination starts are to be considered. In stands of scrub pine (*Pinus virginiana*), loblolly pine (*P. taeda*), shortleaf pine (*P. echinata*), and pitch pine (*P. rigida*) in the vicinity of Washington, D. C., where recently germinated seedlings on bare mineral soils are numerous, search failed to show either seedlings or germinating seed in the litter at points where mineral soil was not exposed. As sufficient moisture was present in the upper layers of the litter, and seed of broad-leaved species was found germinating in fair quantity, suspicion is directed toward some biologic factor as the cause of the lack of conifers. It is suggested that the decay of dormant seed by the vigorous, ordinarily saprophytic mycelia present in leaf mold may prove an important cause of failure to secure natural reproduction of some conifers on humus soils. The entire matter of the importance of the different types of damping-off in limiting reproduction in coniferous forests needs investigation.

INOCULATION PROCEDURE

STANDARD INOCULATION METHOD

Except where otherwise stated, all of the experiments whose results appear in this paper were conducted by the following methods:

Inoculum was added to the soil on which the seed was sown, instead of applying it directly to the seedlings themselves. The procedure was to sow the seed broadcast in pots of a mixture of sand and loam, in most cases containing some compost. The pots and contained soil were sterilized by steam pressure one to three days before seed sowing. The inoculum was laid on the soil at the time seed was sown and both inoculum and seed covered to the depth of about ¼ inch with additional soil or sand, also steamed. Three-inch pots were most commonly used, and all pots received equal quantities of seed, determined in some experiments by count, in some by weight, and in some by volume, or quantities proportional to their areas in the few cases in which pots of different sizes were used in the same experiment. The number of seeds used was in most cases somewhat greater, and in a few cases much greater, than would have been used on an equal area of seed bed in ordinary nursery operations. Partial seed disinfection by sulphuric acid was practiced in a few cases, but not for the most part, as there are no indications that the seed carry parasites to any considerable extent. The inoculum used was the surface layer of an apparently pure culture of the test fungus on prune or corn-meal agar, occasionally on steamed rice or corn-meal mush, and therefore included both nutrient substratum, mycelium, and in most cases spores, except in the case of species of Corticium and Botrytis, which were not observed to form spores on any

of the artificial media used. In typical experiments the inoculum fragments were distributed among the seed over a sector of the pot approximately one-fourth the area of the surface sown. In special cases the inoculum was limited to one or two fragments, while in other experiments fragments were distributed over the entire surface of the soil. In a few of the earlier experiments pots were covered, except during the seed-sowing process, until germination. Stands whose legs were set in pans of water were used in most of the experiments to exclude slugs and ants. In the majority of the inoculations the control pots were given sterile agar from the same lot as that serving as substratum for the cultures placed in the inoculated pots. Results in such experiments were not noticeably different from those in which the controls received no agar. In the later experiments all watering was with heated water, resulting in a decrease in the amount of contamination as indicated by a lesser damping-off occurring in the control pots.

Reisolations, like the original isolations, were made by planting recently affected seedlings in solidified agar plates and transferring from the advancing margins of the resulting growth. Reisolations were made only in experiments in which the control pots had remained free from disease.

DEFICIENCIES IN STANDARD METHOD

The above method of conducting inoculations, involving sterilized soil, heated water, heavy seeding, and heavy inoculation, is a convenient one. The first two of these features are necessary when it is desired to keep the controls entirely free from disease and reisolate the organisms used in the inoculation work. It is the method which has been used by most recent experimenters with root parasites. Attention should, however, be called to the fact that it is not a reliable index of what takes place in ordinary seed beds unless supplemented by experiments under more nearly natural conditions. It is well established that as a substratum for the growth of either higher plants or of fungi steamed soil is a very different thing from normal soil. The quantity of water-soluble matter, both organic and inorganic, is changed; the composition of the organic and inorganic matter is changed; and the effects of destruction of the original microflora and fauna, which can be hardly reestablished for several months in the original composition and balance, can scarcely fail to be reflected upon both hosts and parasites grown in the steamed soil. That the changes due to steaming are of more than theoretical importance is shown by the comparison of results of inoculations on steamed and unsteamed soil. Successful inoculation, at least with some of the parasites, seems much easier to secure in steamed than in normal soil. A further indication that heating soil is likely to abnormally favor damping-off parasites is seen in the heavy spontaneous losses occurring on soil subjected to temperatures of only 80° to 90° C. at some nurseries. In

field tests in the Middle West heated soil plots not specially protected against reinfection have in a number of cases suffered even more heavily from damping-off than plots not treated, despite the demonstrated killing of the parasites originally present in the soil.

Heavy inoculation with material consisting largely of substances like corn-meal mush, rice, and various nutrient agars, as has been customary, undoubtedly results also in soil conditions decidedly different from those which occur in nature. It is believed that the presence of abundant rich food material for the parasites decidedly increases the ability of some of them to attack the seedlings. Heavier seeding than that used in practical seed-bed work has been a part of the experimental procedure in most of the writers' work if not in that of others, and also appears to create conditions abnormally favorable to the parasites. It is felt that all of the work that has been done, including that presented in the following discussion, must be supplemented by further experiments on unsterilized soil, with unheated water, with a density of seed sowing corresponding to that used in regular seed-bed practice, and with inoculum consisting of spore suspensions where practicable, or with small quantities of mycelium in other cases, but in no case with any addition of a nutrient medium. Inoculation in outdoor seed beds, as well as in pots in greenhouses, will be desirable. Conclusive results in such experiments will not always be easy to get. Because of the inevitable damping-off in the controls of experiments so conducted, positive results will have to consist of a heavier damping-off loss than occurs in the controls, a thing which can be demonstrated only by averaging the results obtained from a large number of pots. Until such work is done the relative importance of the various parasites active under field conditions can only be guessed at, no matter how thoroughly parasitism has been demonstrated in experiments of the conventional type.

CORRELATION OF INOCULATION RESULTS

The method of determining the results of inoculations has been to count and remove all damped-off seedlings, making the examinations every two or three days in the case of species like jack pine, which have small seed. Because of the extreme brittleness of the stems of young coniferous seedlings a certain number were accidentally broken in almost every experiment. These were removed as found, and recorded separately. The experiments were ordinarily closed from 10 to 20 days after fairly complete germination had been attained, in order to minimize the effect of accidental contamination on the results; and the surviving seedlings were counted. By adding the number of seedlings lost by damping-off and breakage to those surviving, the total number germinating was secured. By the number germinating is meant not the total germination but rather the number which developed far enough to break through the soil cover, this criterion being the only one it was practicable

to apply. The relative number of seedlings appearing in the inoculated pots and in the controls is an important element in the results, as it is a measure of the seriousness of the germination losses occasioned by the inoculations. It is, of course, not an exact measure because of the unavoidable differences in the number of seeds viable in the different pots at the time of sowing, but it is the only one available.

The effect of the inoculations in causing normal damping-off is best shown by the percentage of the germinated seedlings which damp-off. The absolute number damping-off is not a figure on which conclusions can be based, because of the great variations germination loss may cause in the initial stand. For example, if the pots inoculated with culture A had a germination of only 15 seedlings, the subsequent damping-off of 10 seedlings will mean much more than the damping-off of 20 seedlings in the pots inoculated with culture B, in which 100 seedlings may have originally germinated. As a large proportion of the seedlings lost by mechanical breakage are removed before the major part of the damping-off takes place, it is assumed that part of them would have damped-off had they not been broken. In view of this, the percentage of damped-off seedlings is obtained by the formula

$$\text{per cent damped-off} = \frac{D}{D+S} \times 100,$$

in which D represents the number of seedlings damped-off, and S the number surviving at the time the experiment was closed. The broken seedlings do not enter into this calculation.

The germination and the percentage of damping-off are of value as indicating the seriousness of the germination loss and of the normal damping-off, respectively, but neither figure represents the whole effect of the inoculation. The survival also is therefore given. If the limitations in its exactness due to accidental variations in germination are kept in mind, the comparative survival on inoculated pots and controls can be used as evidence as to the total effect of the inoculations both on germination loss and on normal damping-off. In order to free the survival figures, as far as possible, from the accidental variations due to the different amounts of loss by mechanical breakage in the different pots, it has been assumed that the broken seedlings in any series of pots, had they not been broken and removed, would have lost the same proportion of their number by damping-off as the unbroken seedlings. The actual survival is therefore in all cases adjusted by adding to it the number of the broken seedlings which the damping-off percentage indicates would have survived if they had not been broken. For example, in a set of pots in which the damping-off percentage is 40, the number of broken seedlings 15, and the surviving seedlings 56, the adjusted survival is obtained by adding to the actual survival 60 per cent of the number broken; the adjusted survival is thus 56+9, or 65. In most cases the

number it was necessary to add in adjusting the survival by this method was relatively much smaller than in this sample case.

In cases in which some of the experimental units contained more or larger pots than others, the germination and survival figures for the larger units were reduced proportionally, or were converted for all of the units to a percentage based on the number of seed sown, in order to permit the direct comparison of the figures for the different units.

OOMYCETES CAUSING DAMPING-OFF

At least four oomycetes found in America appear capable of causing the damping-off of pine seedlings. Only one of these, *Pythium debaryanum* Hesse, is believed to be especially important on conifers. *Rheosporangium aphanidermatus* Edson, *Phytophthora* sp., and an apparent species of Pythium with spiny oogones, are the other oomycetes found attacking pines. As some of those concerned are of doubtful identity, the experimental results obtained with them will not be published until further studies now in progress on them have been completed.

CORTICIUM VAGUM

Rhizoctonia was reported as a cause of the damping-off of white pine (*Pinus strobus*) in New York in 1901 (*6*). Preliminary inoculations on conifers were mentioned by the senior writer in an abstract published in 1910 (*13*). No inoculation evidence of its parasitism on conifers has ever been presented.

The strains of Rhizoctonia which were used successfully in the following experiments belonged to the common American Rhizoctonia, which causes the damping-off and rootrot of angiosperms, and is now usually referred to *Corticium vagum* B. and C. (identical with *C. vagum* var. *solani* Burt and with *Hypochnus solani* Prill. and Del.; *21, p. 286, footnote*). Spores have not been produced in any of the writers' cultures, but the identity of the Rhizoctonia on conifers with the common damping-off fungus on angiosperms is considered established by the following facts:

1. Widespread distribution and ready growth on various culture media of the strains from conifers. The only other common American Rhizoctonia, *R. crocorum* (*R. medicaginis; 21*) will not grow on ordinary artificial media (*4*).

2. Successful inoculations on pine seedlings with strains from, or which had been found parasitic on, dicotyledonous hosts.

3. Observed damping-off of dicotyledonous weed seedlings in patches coextensive with definitely limited damping-off patches of pine seedlings.

4. Successful inoculations by Edson (*7*) on dicotyledons with strains which the writers had taken from pines and had found parasitic on them.

Typical mycelium of *Corticium vagum* is easily demonstrated in the tissues of recently killed pine seedlings, and in the soil adjacent to them, and is obtained in culture by planting in solidified prune-agar plates small soil masses, or recently killed seedlings either with or without preliminary washing in mercuric-chlorid solution. Growth on the plates is very rapid, and transfers from the edges of the resulting colony give a large percentage of apparently pure cultures. A distinguishing character of the hyphæ, helpful in conjunction with the typical basal constrictions and septa, but less commonly mentioned, is the lack of tapering in young branches. Young hyphæ are nearly as large as older ones, with thick truncated tips, very unlike the fine tapering tips of some of the other soil fungi whose large hyphæ with basally constricted branches might otherwise be confused with young hyphæ of *C. vagum*.

The coniferous hosts from whose damped-off seedlings *Corticium vagum* has been isolated are western yellow pine[1] and jack pine from Nebraska, red pine (*P. resinosa*) from Minnesota and Michigan, Engelmann spruce from California and District of Columbia, and Douglas fir (*Pseudotsuga taxifolia*) from Colorado. Cultures from all of these hosts, with the exception of Douglas fir, have proved parasitic on pine seedlings. The single strain isolated from Douglas fir proved weakly if at all parasitic; so many infections have been observed in seedlings of this fir that it is believed some of them have been caused by parasitic strains. The fungus was also seen in the tissues of, or in cultures from, damped-off seedlings of Scotch pine (*Pinus sylvestris*), Corsican pine, Austrian pine (*Pinus austriaca*), and Norway spruce (*Picea excelsa*) without attempting to isolate it. With the previously reported findings of the fungus in damped-off white pine (*6*), yew (*Taxus cuspidata; 1*), and *T. canadensis*,[2] the number of coniferous hosts on which *C. vagum* apparently causes damping-off in nature is raised to 12, and will probably be much further increased when other conifers are studied.

Rhizoctonia has also been found associated with needle-killing of white pine (*2*) and Douglas fir (*14*) in seedlings more than 1 year old.

INOCULATIONS ON AUTOCLAVED SOIL

Inoculations by the standard method described on page 526 were made with strains of *Corticium vagum* on conifers in the autumn of 1909, and repeated in later years. Inoculations with agar cultures broadcast at one side of the pot were made on jack pine in two experiments and on both red and western yellow pines in two other experiments, germination being reduced in the inoculated pots, and damping-off appearing, while the controls, with the exception of the red pine in the last experi-

[1] All of the western yellow pine mentioned in this paper was from seed collected in South Dakota, Colorado, or New Mexico, and is therefore the small-seeded form of *Pinus ponderosa* Laws (*P. scopulorum* Lemmon).

[2] The report of this latter species as a host for *Corticium vagum* is attributed to Clinton by Peltier (*21, p. 304*), though it does not seem to be mentioned by Clinton in (*1*).

ment, remained free from disease. With the last two hosts the controls received sterile agar.

In five additional experiments inoculum was distributed broadcast over the entire pot. In three of these, cultures on corn-meal mush were used as inoculum, and most of the germinating seed of jack and western yellow pine, and Douglas fir, respectively, were killed before they were able to break through the soil. The total number of seedlings involved in the experiment with Douglas fir was small. Though the controls were treated with sterile corn-meal mush and though the fungus was found in the killed seedlings in the inoculated flat, repetition is considered desirable for this host. In the two other experiments positive results were secured with both of the pines, with agar cultures as inoculum.

In five other experiments positive results were obtained with these pines by inoculating them with agar cultures at only one or two points in each pot and in one of them very definite results were also secured with white pine. In two of these experiments on jack pine, inoculation with single sclerotia in each pot was tried without the addition of any of the nutrient substratum with entire success. In the experiments with sclerotial inoculation and in the test on white pine the controls remained entirely free from disease. In the other experiments mentioned in this paragraph more or less accidental infection took place in the controls; positive results consisted in less germination and more subsequent damping-off in the treated plots than in the controls. In the inoculations mentioned in this and the preceding paragraphs the number of pots inoculated with *Corticium vagum* varied from a single 10-inch flat in the smallest experiment to 178 3-inch pots in the largest; the controls from a single flat to 30 pots.

In only two experiments *Corticium vagum* failed to give positive results. The loss was heavier in the inoculated pots than in the controls in both cases, but the difference was so slight as to be negligible. Both of these experiments involved inoculation at two points only in each pot.

These inoculations have the effect of confirming the field evidence of the parasitism of *Corticium vagum* on four pines, jack, red, white, and western yellow, and somewhat less positively on Douglas fir. Its apparent success on all of the coniferous hosts tested justifies the prediction that under favorable conditions it is likely to be found to be one of the causes of damping-off of most or all of the coniferous species which commonly suffer from this disease. Only part of the strains are vigorously parasitic on conifers, some strains, even though isolated from conifers, failing in repeated inoculation tests under favorable conditions to produce any considerable amount of disease.

REISOLATION AND REINOCULATION

To complete the proof of the parasitism of *Corticium vagum* reisolations were made from recently killed seedlings of jack and western yellow pine in pots which had been inoculated with the fungus in an experiment in which the controls had remained free from disease. The results in the initial experiment and in two subsequent experiments conducted at different times in which the reisolated strains were used are given in Table I.

TABLE I.—*Inoculation with initial and reisolated strains of Corticium vagum on pine seedlings in autoclaved soil*

Experiment No. and date.	Strain No.	Source.	Trial host.	Number of pots.	Location of inoculum.	Results.		
						Germinated.	Damped-off.	Survival.
						Per 5 pots.	*Per cent.*	*Per 5 pots.*
58, fall, 1915.	147	Damped-off Engelmann spruce seedlings.	Western yellow pine.	5	Agar cultures at 1 point per pot.	28	68	9
Do	do	do	Jack pine	5	do	18	39	11
Do	[a] 213	Damped-off sugar-beet seedlings.	do	5	Agar cultures at 6 points per pot.	1	100	0
Do	230	Russian wild-olive seedlings.[b]	do	5	Agar cultures at 8 points per pot.	4	100	0
Do	Control		Western yellow pine.	5	None	55	0	55
Do	do		Jack pine	10	do	78	0	78
						Per 3 pots.		*Per 3 pots.*
71 and 72, fall, 1917.	147	Damped-off Engelmann spruce seedlings.	Western yellow pine.	2	Agar cultures; fragments scattered over one side of pot.	2	0	2
				3		0	.....	0
Do	329	Reisolation of No. 147 from western yellow pine, experiment 58.	do	3	do	0	.....	0
				2		0	.....	0
Do	332	do	do	3	do	3	100	0
				2		0	.....	0
Do	333	do	do	2	do	0	.....	0
				2		0	.....	0
Do	343	Reisolation of No. 147 from jack pine, experiment 58.	do	3	do	0	.....	0
				2		0	.....	0
Do	[a] 213	Damped-off sugar-beet seedlings.	do	3	do	0	.....	0
				3		0	.....	0
Do	340	Reisolation of No. 213 from jack pine, experiment 58.	do	2	do	3	100	0
				3		0	.....	0
Do	233	Russian wild-olive seedlings [b] (duplicate of No. 230).	do	1	do	9	0	9
				2		16	8	16.5
Do	330	Reisolation of No. 230 from jack pine, experiment 58.	do	1	do	9	0	9
				3		8	0	8
Do	341	do	do	3	do	1	100	0
				3		6	33	4
Do	Control		do	4	Sterile agar fragments scattered over one side of pot.	9	0	9
				14		9	0	9
Do	147	Damped-off Engelmann spruce seedlings.	Red pine	2	Agar culture fragments scattered over one side of pot.	6	100	0
				2		0	.....	0

[a] Cultures furnished by Dr. H. A. Edson, Bureau of Plant Industry.
[b] Diseased material furnished by Mr. B. R. H. d'Allemand.

TABLE I.—*Inoculation with initial and reisolated strains of Corticium vagum on pine seedlings in autoclaved soil*—Continued

Experiment No. and date.	Strain No.	Source.	Trial host.	Number of pots.	Location of inoculum.	Results.		
						Germinated.	Damped-off.	Survival.
						Per 3 pots.	*Per cent.*	*Per 3 pots.*
71 and 72, fall, 1917.	329	Reisolations of No. 147 from western yellow pine, experiment 58.	Red pine.....	2	Agar culture fragments scattered over one side of pot.	14	11	12
				3		2	100	0
Do...	332	.....do.....	.....do.....	3	.....do.....	2	100	0
				3		0	.....	0
Do...	333	.....do.....	.....do.....	2	.....do.....	8	100	0
				3		0	.....	0
Do...	343	Reisolation of No. 147 from jack pine, experiment 58.	.....do.....	3	.....do.....	2	0	2
				3		0	.....	0
Do...	[a] 213	Damped-off sugar-beet seedlings.	.....do.....	3	.....do.....	5	100	0
				3		0	.....	0
Do...	331	Reisolations of No. 213 from jack pine, experiment 58.	.....do.....	2	.....do.....	3	100	0
				2		0	.....	0
Do...	340	.....do.....	.....do.....	3	.....do.....	4	75	1
				3		1	100	0
Do...	230	Russian wild-olive seedlings.	.....do.....	3	.....do.....	58	2	57
				2		33	5	31.5
Do...	330	Reisolations of No. 230 from jack pine, experiment 58.	.....do.....	3	.....do.....	65	3	63
				3		41	0	41
Do...	341	.....do.....	.....do.....	3	.....do.....	31	45	17
				2		30	30	30
Do...	Control	.....	.....do.....	5	Steriie agar fragments scattered over one side of pot.	58	0	58
				16		35	5	33

[a] Cultures furnished by Dr. H. A. Edson, Bureau of Plant Industry.

It is evident from the results in Table I that the reisolated cultures were able to cause germination loss or damping-off, or both, in both the hosts on which they were tried. The approximate agreement in virulence in experiment 71 and 72 of the original strains and the strains isolated from the pots inoculated with them is an additional evidence that the strains recovered were the ones originally used. Strain 341 seemed rather more virulent than 230 from which it was reisolated; it was, nevertheless, obviously less active as a parasite than strains 147 and 213, and their reisolations. The possibility that a strain of *Corticium vagum* obtained from an autoclaved and inoculated pot is not a true reisolation of the strain used in the initial inoculation is very slight, in view of the lack of adaptation of the fungus to aerial dissemination and the fact that in the writers' numerous cultures from autoclaved soil experiments, *C. vagum*, unlike *Fusarium* spp. and *Pythium debaryanum*, has never been detected in pots in which it had not been intentionally introduced. As the cultures used were all, or nearly all, obtained by the planted-plate method, their purity is not entirely beyond question. However, their apparent purity, continued in the case of strain 147 through eight years of growth on artificial media, and the permanence of the relative

virulence of most of the strains through several years cultivation, indicate not only that the cultures used contained only *C. vagum*, but that most of them contained only a single strain of the fungus.

On the whole, the limited comparisons possible of initial and reisolated strains, do not indicate any decided increase in virulence on pine seedlings as a result of a single passage through the host.

CROSS-INOCULATIONS

In Table I is presented evidence of parasitism on three species of pine of strains of *Corticium vagum* from spruce and sugar beet. In one of these experiments and also in an earlier experiment not included in this table, the original strains from Russian wild olive (*Elaeagnus sp.*), proved decidedly parasitic on jack pine. The strains 147 and 213, from spruce and sugar beet, respectively, have proved more virulent on the pines in these and other experiments than any of the strains coming originally from pine seedlings. The slight ability of a single strain from Douglas fir to infect pine, referred to earlier, does not prove any specialization of virulence, as some of the strains from pine give negative results on the same species of pine unless given very favorable working conditions. The positive results on Douglas fir with a strain from jack pine in a single very small-scale test made is indication of the lack of any specialization of strains of *C. vagum* to pine or Douglas fir.

Inoculations on both jack and western yellow pine have been successful with strains from potato tuber and from bean stems, the latter culture being furnished by Dr. M. F. Barrus, who stated that it was from a strain of proved parasitism on beans. The strains from these two hosts were parasitic on pines only under very favorable conditions. Single experiments with strains from alfalfa and carnation supplied by Dr. Barrus, indicated that these were only weakly, if at all, parasitic on pine seedlings. A strain from a sugar-beet root, isolated in eastern Colorado by Dr. F. A. Wolf, of the North Carolina Experiment Station, proved moderately parasitic on pine seedlings, while another strain from the same host and locality showed little, if any, virulence on pine even under the most favorable conditions. All three of the sugar-beet strains had been previously tested by Dr. Edson in his inoculations on sugar-beet seedlings, and found parasitic on them. Cultures from western yellow pine were also tested on sugar-beet seedlings by Edson (7) with positive results. A strain from jack pine, only moderately parasitic on that pine species in inoculation, was found by Dr. Barrus to produce lesions on bean stems, though small and atypical as compared with his own strains.

The inoculation evidence, as a whole, supports the conclusion that the Rhizoctonia causing the damping-off of pines is the same as the Rhizoctonia commonly concerned in the seedling disease of dicotyledons. The same strain can cause disease of both conifers and dicotyledons. Cer-

tain strains may be slightly specialized to particular hosts, but such variations are hardly of taxonomic value.

INOCULATIONS ON UNSTERILIZED SOIL

In an experiment in which a very sandy western Kansas soil was treated with 0.5 fluid ounce of sulphuric acid per square foot, followed two days later by 0.9 ounce of air-slaked lime per square foot, a number of strains of *Corticium vagum* were tested, inoculum on cooked rice being distributed through the drills. Most of the strains either prevented or greatly decreased the germination of both jack and western yellow pines, and the more virulent strains caused the damping-off of practically all of the seedlings which were able to get through the soil surface. In the plots inoculated with strains of *C. vagum* which had indicated any decided degree of virulence in earlier tests the survival ranged from 0 to 47 seedlings (44 linear inches of drill, half of each pine, for each fungus strain), while the 16 controls in the same experiments ranged from 59 to 254 seedlings for equal lengths of drill, more than half of the controls having survivals better than 150 seedlings. The most virulent strain of *Pythium debaryanum* heavily inoculated in the same way and at the same time resulted in little more parasitic loss than occurred in control plots inoculated with saprophytic molds or with nothing at all. The inoculations with *Corticium vagum* caused as much damping-off as would normally be secured by inoculating autoclaved soil under the most favorable conditions for parasitism. The advantage of the *C. vagum* over the *Pythium debaryanum* in this case was probably at least in part due to the lack of humus. *P. debaryanum* apparently prefers soils with a reasonable amount of humus, while *C. vagum* has been frequently found in damped-off seedlings on this same soil and on another very sandy, humus-poor soil.

In an experiment in which a heavier soil heated in a moist condition to a temperature of 80° to 90° C. for a period of not less than 10 minutes was inoculated with the parasites. *Corticium vagum* again caused marked decreases in germination, while *Pythium debaryanum* had little effect on the number of seedlings which appeared. Outside infections, probably with *Fusarium* spp., which it was impossible to exclude in this outdoor work, destroyed the seedlings so rapidly after germination that it was impossible to obtain evidence on the effect of the inoculum on the seedlings after they came up. The definite superiority of *C. vagum* over the *P. debaryanum* in causing germination loss in this case can not be attributed to lack of humus.

In soil entirely untreated, *Corticium vagum* was used in two very small-scale inoculation experiments in the Washington greenhouse, inoculum being placed at one and two points in each pot, respectively. In both cases *C. vagum* definitely decreased the survival. In the first experiment *Pythium debaryanum* had no effect, and in the second affected only germination, while *C. vagum* both decreased germination and increased

subsequent damping-off. The soil used was a greenhouse mixture of sand and loam.

FUSARIUM SPP.

Species of Fusarium and Fusoma have been frequently reported as being the cause of damping-off of conifers in Europe. The first crude inoculations were made by Hartig (*12*), who produced typical damping-off by placing healthy seedlings in contact with plants which had damped-off and were bearing spores of *Fusarium* spp. Von Tubeuf (*27*) reports having inoculated pine seedlings with artificial cultures, but in so small an experiment and with so little in the way of positive results as to be inconclusive. He later (*27*) states that he and Hartig have repeatedly caused the death of plants of European conifers by inoculating them with *Fusarium parasiticum*, "also from pure cultures." No detailed report of pure-culture inoculation experiments has been furnished in the European literature noted, and the ability of the Fusarium or Fusoma strains, which have been variously mentioned under the specific names of *pini*, *parasiticum*, and *blasticola* to cause damping-off in Europe has rested mainly on the frequency with which they have been obtained from damped-off conifers. The strains which have been found on conifers and which have been used in inoculation are not sufficiently well described to make it possible to connect them with any of the species at present recognized. There is furthermore no way of telling whether the different reports of species of Fusarium and Fusoma on coniferous seedlings refer to the same or different organisms.

In America Spaulding (*25*) reports briefly inoculation experiments with a number of different strains of Fusarium the detailed record of which the writers have been permitted to examine. Sufficient damping-off occurred in some of the control plots, so that it does not seem possible to say for any one of the strains he used that its parasitism was proved, in view of the fact that each strain was used in a single pot only. For the strains in general, his work established beyond reasonable doubt that at least some of them were parasitic, the loss in the pots inoculated with *Fusarium* spp. as a whole averaging well above that in the controls. Among the species for which parasitism was indicated quite strongly are *F. vasinfectum* E. F. Smith and *F. moniliforme* Sheldon. The evidence is especially strong for the latter species. *F. vasinfectum* from cotton gave apparently more positive results than the strain from watermelon. The general conclusion from his experiment seems to be that a number of different strains or species of Fusarium are probably able to attack pine seedlings under the very favorable inoculation conditions which he furnished, and that *F. moniliforme* is one of the more virulent.

Prof P. S. Lovejoy, working as a student under the direction of Dr. J. B. Pollock in 1907, two years after Spaulding did his work, produced damping-off in western yellow pine with a species of Fusarium isolated from

damped-off pine seedlings. His experiments were conducted in autoclaved earth, with several pots both inoculated and controls, all being under bell-jars. The controls remained free from disease. His species of Fusarium, which he called *F. pini*, can not be positively identified with any of the species at present recognized, from the data given in his unpublished manuscript, which he kindly allowed the writer to examine. A species of Nectria (later reported by Pollock; *22*) was associated with the species of Fusarium.

Gifford also carried out inoculation experiments with species of Fusarium indicating its parasitism. His statement (*9, p. 157*) seems reasonably conclusive, though the tables on the pages preceding and following, presumably by reason of typographical errors, do not bear out his statement as to the original disease freedom of his control pots. The host with which he experimented was Scotch pine. The description of the fungus which he gave indicates that it is not *F. moniliforme*, but does not make it possible to refer it to any of the other recognized species. While his inoculations were on autoclaved soil, the fungus was not given quite such optimum conditions as in Spaulding's work, in that his inoculum consisted of a spore suspension without any considerable amount of nutrient medium accompanying.

FUSARIUM MONILIFORME

The species of Fusarium which the writers have found most virulent among the four or more species which they have tested is *F. moniliforme*[1] This fungus is fortunately easy to distinguish from other species of the genus, as it is understood to be the only species of Fusarium, in the United States at least, which forms its microspores in chains. It is presumably not identical with the species and relatives of Fusarium described as troublesome to coniferous seedlings in Europe, as none of the European reports noted mentioned moniliform spores. The microspores in the writers' cultures were ordinarily produced in delicate long unbranched chains. Presumably on account of the delicacy of these spore chains, the fungus seems especially well adapted for aerial dissemination. In planted petri-dish cultures numerous new colonies usually start well in advance of the original colony, before the mycelium from the original inoculation is able to cross the plate. In some cases, as in Sheldon's cultures (*23*), microspores were agglomerated into heads, and occasionally small heads of spores with chains arising from them have been observed. The spores in the chains measured in one case from a prune-agar culture 2.2 to 3.1 by 4.8 to 6.3 μ. In another case from a corn-meal agar culture 27 of the microspores ranged in length from 7.4 to 11.1 μ. Many of the cultures on the above media, but not all, developed

[1] All positive Fusarium identifications were made by Mr. C. W. Carpenter, Plant Pathologist of the Hawaiian Experiment Station, based on comparisons with stock cultures named by Dr. W. H. Wollenweber, formerly of the Bureau of Plant Industry.

a characteristic grape-juice purple, which diffused through the agar. This was not constant even for the same strain. The moniliform character of the microspores of this species can be very easily demonstrated by growing the fungus on a thin layer of nutrient agar in a petri dish and examining the culture from above with the compound microscope after four or five days' incubation at room temperature.

The cultures used by the writers were isolated from seedlings of jack and western yellow pine from nurseries on somewhat alkaline and very sandy soils in southwestern Kansas. It is not believed to be a common organism on pine seedlings in most localities.

One or more strains of *Fusarium moniliforme* were tested in five inoculation experiments on jack pine in autoclaved soil. In two of these experiments, inoculation was only light or moderately heavy, and the pots inoculated with *F. moniliforme* suffered distinctly less from damping off than the controls, which were rather seriously affected as a result of accidental contamination. A virulent strain of *Corticium vagum* also proved a failure in one of the experiments, and the most virulent strains of Corticium and Pythium were only slightly active in the other. The results in the three remaining experiments, together with the results from all the other fungi which were used in the same experiments, are given in Table II, with the exception that the results of inoculation with strains of Corticium, which were atypical or intermediate in virulence, in experiment 31 are omitted. The results of the two larger experiments seem to establish beyond any serious doubt the ability of *F. moniliforme* to cause damping-off of jack pine on autoclaved soil when sufficient inoculum is added. Absolute final proof must, of course, await compliance with Koch's postulates and should be based on inoculation with single spore cultures. The cultures used in these experiments were all from planted plates. Nevertheless all of those whose results are reported were apparently pure, and in view of the number of strains and of controls in experiments 31 and 60, the parasitism of the fungus under favorable conditions is considered practically established. In addition to the pots formally designated as controls, the 35 pots inoculated with *Trichoderma* sp. and with unidentified or mixed cultures in experiment 31 serve perhaps as still better controls, as they received in the inoculum the same nutrient medium as was applied to the pots inoculated with *F. moniliforme*.

TABLE II.—*Inoculation with Fusarium spp. and other fungi on jack pine in autoclaved soil*

Experiment No. and date.	Nutrient substratum introduced with inoculum.	Location of inoculum.	Number of pots.	Fungus.	Germination (per-centage of seed).	Damped-off (per-centage of seed-lings).	Survived (per-centage of seed).
31, fall, 1913.	Prune agar and steamed rice.	Broadcast through-out pot.	5	*F. moniliforme* (strain 239).	44.1	36.0	28.8
Do.	do.	do.	5	*F. moniliforme* (strain 249).	35.8	48.0	18.6
Do.	do.	do.	5	*F. moniliforme* (strain 251).	46.0	90.0	4.7
Do.	do.	do.	5	*F. moniliforme* (strain 260).	49.3	53.0	23.7
Do.	do.	do.	5	A species of Fusarium somewhat like *F. solani* (strain 235).	41.5	9.0	38.0
Do.	do.	do.	5	A weak strain of *Pythium debaryanum* (258)	36.9	18.0	30.6
Do.	do.	do.	5	A virulent strain of Corticium (147).	2.9	73.0	.7
Do.	do.	do.	5	*Trichoderma* sp. (252)	51.1	2.7	49.5
Do.	do.	do.	30	Unidentified or mixed cultures (strains 244, 250, 236, 238, 246, 248).	48.1	4.6	45.9
Do.	Steamed rice	do.	5	*F. ventricosum*	40.0	38.0	25.2
Do.	Corn-meal agar and prune agar.	do.	5	*F. solani*	45.4	14.0	39.0
Do.	Corn-meal agar	do.	5	*F. acuminatum*	55.5	2.2	54.2
Do.	do.	do.	5	*Rheosporangium aphanidermatus* (229)[a].	45.3	13.0	40.0
Do.	do.	do.	5	*Phoma betae*[a]	48.6	5.6	45.9
Do.	do.	do.	5	*Chaetomium* sp.	45.7	6.2	42.8
Do.	do.	do.	5	*Trichothecium roseum*	48.2	3.4	46.3
Do.	Prune agar	do.	5	A virulent strain of *P. debaryanum* (255).	12.4	44.0	6.8
Do.	do.	do.	10	The most virulent strains of Corticium (213,[a] 233).	3.9	60.0	1.3
Do.	do.	do.	10	The 2 least virulent strains of Corticium (205, 186).	44.5	4.7	42.4
Do.	do.	do.	5	*Phoma* sp.	50.0	3.4	48.3
Do.	None		25	Controls	43.0	5.2	40.8
57, fall, 1914.	Autoclaved Melilotus stems.	Spore suspension after sowing and 5 small pieces of stem inoculum in pot at germination.	8	*F. moniliforme* (249)	37.7	5.3	35.8
Do.	Corn-meal agar	2 points at one side of pot.	7	*P. debaryanum* (295)	38.8	1.8	38.2
Do.	do.	do.	5	*Corticium vagum*	37.3	10.3	33.5
Do.	None		12	Controls	42.0	.4	41.8
					Per 5 pots.	*Per 5 pots.*	*Per 5 pots.*
60, 1915.	Prune agar	1 point at edge of pot.	5	*F. moniliforme* (260)	54.0	6.0	51.0
Do.	do.	4 points in pot	5	do.	50.0	32.0	34.0
Do.	do.	Broadcast at one side of pot.	5	do.	28.0	57.0	12.0
Do.	do.	Broadcast through-out pot.	5	do.	40.0	35.0	26.0
Do.	Steamed rice	do.	10	do.	22.5	42.0	13.0
Do.	Prune agar and steamed rice.	do.	5	do.	4.0	50.0	2.0
Do.	None (spore suspension).	do.	(1st unit) 5.	do.	46.0	4.3	44.0
Do.	do.	do.	(2d unit) 5.	do.	51.0	43.0	29.0
Do.	Prune agar	1 point at edge	5	*Fusarium* sp. (273)	59.0	5.1	56.0
Do.	do.	4 points in pot	5	do.	47.0	4.3	45.0
Do.	do.	Broadcast at one side of pot.	5	do.	53.0	1.9	52.0
Do.	do.	Broadcast through-out pot.	5	do.	44.0	18.0	36.0
Do.	Steamed rice	do.	10	do.	46.0	21.0	34.5

[a] Furnished by Dr. H. A. Edson.

TABLE II.—*Inoculation with Fusarium spp. and other fungi on jack pine in autoclaved soil*—Continued

Experiment No. and date.	Nutrient substratum introduced with inoculum.	Location of inoculum.	Number of pots.	Fungus.	Germination (percentage of seed).	Damped-off (percentage of seedlings).	Survived (percentage of seed).
60, 1915..	Prune agar and steamed rice.	Broadcast throughout pot.	5	*Fusarium* sp. (273)....	33.0	15.0	28.0
Do....	None (spore suspension.)	.....do.....	5. (1st unit)	.....do.....	49.0	6.0	46.0
Do....	.....do.....	.....do.....	5. (2d unit)	.....do.....	58.0	1.7	57.0
Do....	Prune agar and steamed rice.	.....do.....	5. 5	*F. solani* (202).....	20.0	40.0	12.0
Do....	None.....	.....	5. (1st unit)	Control (*a*).....	57.0	7.0	53.0
Do....	.....do.....	.....	5. 2d unit)	Control (*b*).....	55.0	7.0	51.0
Do....	.....do.....	.....	5. (3d unit)	Control (*c*).....	48.0	0.0	48.0
Do....	.....do.....	.....	5. All.	Control (*a*, *b*, *c*).....	53.3	5.0	50.7

The peculiar behavior of *Fusarium moniliforme* in actually seeming to prevent part of the damping-off in the first two experiments mentioned (the two which are not included in Table II) suggests the possibility that in autoclaved soil under circumstances in which it is not itself able to act as a parasite, it may nevertheless by reason of its vigorous saprophytic growth so occupy the soil as to make it a less favorable medium for the spread of the more virulent parasite or parasites whose presence was indicated by the considerable damping-off in the controls in these experiments. That competition of saprophytic fungi may limit the damage done by *Pythium debaryanum* in recently autoclaved soil is indicated by the results of counterinoculation experiments which will soon be published. Competition between parasites, or between a parasite and a potentially parasitic fungus under conditions which render the latter nonparasitic, is quite probable. In the present case the record of this species of Fusarium in killing other fungi in mixed cultures in agar adds color to the possibility that it may effectively hinder more virulent parasites in autoclaved soil.

In experiment 60 it appears that in heavy inoculations with cultures on nutrient substrata *Fusarium moniliforme* caused germination loss as well as damping-off after the seedlings appeared. This agrees with Spaulding's conclusions (*26*). It is apparently only under exceptionally favorable conditions such as very heavy inoculation, or, as in Spaulding's work, unusually deep sowing, that serious germination loss is to be expected from this species of Fusarium. Comparison of the Fusarium pots inoculated with *F. moniliforme* with those inoculated with virulent strains of

Pythium and Corticium in experiment 31 indicates much less damage by *F. moniliforme* to germinating seed. While some of the strains of Corticium and Pythium also do little or no damage to germinating seed in inoculations by the writer's standard methods, they are in most cases strains whose general virulence is low, and which are not able to kill many seedlings after germination. The indications are that *C. vagum* is rather better able to kill germinating seed than seedlings which have recently appeared above the soil; that *P. debaryanum* is at least approximately as well able to cause germination loss as normal damping-off; and that *F. moniliforme* is distinctly less able to cause germination loss than it is to cause subsequent damping-off.

The relation of heavy inoculation to positive results with *F. moniliforme* is indicated, first, by the negative results in the two nontabulated experiments in which inoculation was light, the weakly positive results in experiment 57 in which inoculation was fairly heavy, and the unquestionable results in the heavier inoculations in experiments 31 and 60; and second, by the difference in results between the heavily inoculated and the parallel lightly inoculated pots in experiment 60. It is not possible to say whether the increased damping-off in the heaviest inoculations was simply due to the development in the soil of a larger amount of mycelium and consequently more points of contact between hyphæ and seedlings, or whether in the cases in which large amounts of nutrient substratum were added with the fungus, there was an actual temporary increase of virulence resulting. It appears from experiment 60 that broadcast inoculations with spore suspensions were distinctly more effective than inoculation with a small fragment of a culture at a single point at the edge of each pot. The single point inoculations resulted in no more damping-off than occurred in the controls. Broadcast inoculation over the entire pot and including nutrient substrata were definitely successful in all four of the 5-pot units on which it was used. These heavy inoculations were clearly more effective tlan the inoculations over smaller areas or those made with spore suspensions. In comparing these heavily inoculated units with each other and considering both germination loss and subsequent damping-off, it appears that inoculation with cultures on steamed rice is more effective than with cultures on prune agar, and that the greatest total loss occurred following the use of both media in the inoculum. The apparent increase in damping-off with increase in the amount and richness of the media, though by no means final proof, is believed to indicate that the presence of the nutrient substrata actually increased the virulence of the parasite, as well as assisting it to become thoroughly distributed throughout the pot. The possibility that the substrata added may have decreased the resistance of the seedlings must also, of course, be considered.

In the series of outdoor inoculations on a Kansas sand lacking humus and treated with sulphuric acid followed by lime, mentioned in the foregoing account of *Corticium vagum*, three strains of *Fusarium moniliforme* were tested, each being added to 2 plots of jack pine and 2 plots of western yellow pine. The average germination for all 12 inoculated plots was only 0.7 that in the 16 nearest control plots, and subsequent damping-off was slightly greater than in the controls. The net results indicate much less parasitism by *F. moniliforme* under these conditions than by most of the Corticium strains used in the same experiment, although the species of Fusarium appears nearly or quite as parasitic as the cultures of *Pythium debaryanum* used. The variation in the controls in the parts of the experiments containing the Fusarium plots was unusually great, and the results less conclusive than those for *P. debaryanum*.

The virulence of different strains of *Fusarium moniliforme* apparently differs (Table II, experiment 31), though the range of variation is less for the four strains worked with than was found to be the case in the larger number of strains of *Pythium debaryanum* and *Corticium vagum*. The weakest strain was more virulent than the weakest strains of these two fungi, but the most virulent strains of *F. moniliforme* were considerably less destructive to jack pine than the most virulent strains of the two better known parasites, at least on the youngest seedlings. No direct comparison of virulence is possible between three fungi whose different strains vary so decidedly among themselves, unless large numbers of strains of all three from different sources are studied. It seems safe, however, to say that *F. moniliforme* is distinctly less important than *P. debaryanum* or *C. vagum* as a parasite on pine seedlings, in view of its apparent infrequence at most nurseries and its failure to cause serious damping-off in inoculation tests, except with very heavy inoculations.

FUSARIUM VENTRICOSUM

A species of Fusarium obtained in association with *F. moniliforme* on an apparently healthy root of western yellow pine was identified by Mr. C. W. Carpenter as *F. ventricosum* Appel and Wollenw. In inoculation on jack pine (Table II, experiment 31) the fungus showed decided evidence of parasitism. Like *F. moniliforme*, in the same series, it had no marked effect on germinating seed, but caused subsequent damping-off loss more than seven times as great as that which occurred in the uninoculated pots, and eight times as great as that in pots inoculated with various saprophytic organisms on the same nutrient medium. There seems little question as to its parasitism, though further tests, involving single-spore cultures and reisolation, will be needed for final proof. How frequently it occurs in pine seed beds is not known; it is, however, believed not to be especially common. Its indicated virulence in the test mentioned was a little below that of *F. moniliforme*.

FUSARIUM SOLANI

A species of Fusarium obtained by Mrs. H. E. Watkins from damped-off western yellow pine in Nebraska was determined by Mr. C. W. Carpenter as *F. solani*. In a single-pot test on jack pine in autoclaved soil it gave some indication of parasitism, but very much weaker than any of the *Pythium debaryanum* strains used in the same experiment. In a later experiment (No. 31, Table II) it gave definite indication of a slight degree of parasitism, both on comparison with the uninoculated controls and with the numerous pots inoculated with species of Phoma, Trichothecium, Trichoderma, Chaetomium, nonvirulent Corticium strains, and other fungi, in all of which damping-off was less than half that in the *F. solani* pots. Though comparison with the other parasites is somewhat uncertain because of the different nutrient substrata used in the inoculum, *F. solani* appears distinctly less parasitic than the stronger strains of Pythium and Corticium (No. 255, 147) and those of *F. moniliforme* and *F. ventricosum* tested, while about equal in effectiveness to a weak Pythium strain and to *Rheosporangium aphanidermatus*. In experiment 60 of Table II heavy inoculation with *F. solani* resulted in decided germination loss and subsequent damping-off, though less than that indicated for *F. moniliforme* with the same inoculum. The absence of nutrient substratum in the control pots prevents the experiment being quite as conclusive as it might have otherwise been.

In inoculation tests on jack pine and western yellow pine on soil treated with acid and lime, referred to in preceding sections, it failed to affect either germination or subsequent damping-off, while all but the very weakest strains of Corticium caused greatly increased losses, and *Pythium debaryanum* and *Fusarium moniliforme* appeared to have moderately increased loss.

The experiments as a whole indicate that the strain of *Fusarium solani* used in these experiments is a weak parasite on jack pine. Despite the relatively slight virulence of this strain, this species seems worth serious consideration as a damping-off organism, in view of its widespread occurrence.

OTHER SPECIES OF FUSARIUM

A preliminary test was made of a mixture of three of Spaulding's cultures of *Fusarium* spp. and three obtained by the writers from pine seedlings at Halsey, Nebr., the latter superficially resembling *F. solani* in heavy inoculation with rice inoculum on jack pine on autoclaved soil and under moist chamber conditions. Damping-off was negligible. In the first test mentioned under *F. solani*, another strain of Fusarium resembling it was also tested on a single pot, with less resultant damping-off than in the control pots; three strains of *Pythium debaryanum* in the same series all caused heavy and unmistakable increases in damping-off.

In experiment 31, Table II, another strain of Fusarium superficially resembling *F. solani* was tested in heavy inoculation on five pots of jack pine. Damping-off after germination was nearly double that in the uninoculated controls and quite double that in pots inoculated with saprophytic molds on the same substrata. The loss, however, was only two-thirds as much as in the pots inoculated with *F. solani*, *Rhcosporangium aphanidermatus*, and weak *Pythium debaryanum*, and much less than in the pots inoculated with the other parasites. The same culture, tested later in the aforementioned experiments on soil treated with acid and lime, gave no indications of parasitism on western yellow pine and little, if any, on jack pine. In all the experiments with this culture the difference between the inoculated pots and the controls was easily within the limits of accidental variation.

A species of Fusarium obtained from damped-off seedlings of western yellow pine in a greenhouse at Washington, D. C., and identified by Mr. Carpenter as *F. acuminatum* E. and E., was tested in experiment 31, Table II, with less subsequent damping-off than in the controls. In the experiment on acid-lime-treated soil it had no apparent effect on yellow pine and on jack pine seemed to increase the amount of damping-off after germination but not more than might be explained on the basis of accidental variation.

A culture of *Fusarium* sp. of uncertain identity (No. 273, experiment 60, Table II) gave positive inoculation results in very heavy inoculations only. This culture was recorded in early notes as producing microspores in chains, but it was impossible to confirm this in subcultures made some time later. In the experiment this culture appeared not only much less virulent than the *F. moniliforme*, but even less so than the *F. solani* culture tested. In view of its doubtful identity and purity, the results with it are of interest chiefly in its agreement with the results in the pots of *F. moniliforme*, broadcast inoculation proving more effective than inoculation over limited areas, agar-culture inoculum proving more effective than spore suspensions, and rice inoculum appearing perhaps still more effective, while the very heavy inoculation involved in the use of both media had the maximum effect.

The conclusion to be drawn from these somewhat fragmentary data is that the strains of *Fusarium moniliforme* and *F. ventricosum* experimented with were above the average of virulence for species of Fusarium on pine seedlings, and that finding a species of Fusarium on damped-off seedlings does not establish as strong a presumption of etiological significance as would the finding of *P. debaryanum* or *C. vagum*. *F. solani* and *F. vasinfectum* also appear somewhat parasitic on pine seedlings, and *F. acuminatum* nonparasitic. Further tests with some of these and other authentically identified species of Fusarium are badly needed to settle the question as to which species are capable of parasitism and

what is their importance as compared with the parasites belonging to other genera, under natural soil conditions.

TRICHODERMA.

Strains of Trichoderma frequently appear in cultures from seedlings affected with the usual type of damping-off. They also often fruit on the surface of recently autoclaved soil, entirely independently of the presence of seedlings. Strains isolated at a Kansas nursery produced conidiophores in very compact groups, often as much as 2 mm. in diameter, and usually arranged in zonate rings. The spores were mostly spherical. This agrees with the characters given for *T. lignorum* (Tode) Harz., though one of the zonate strains isolated had the elliptical spores described by Cook and Taubenhaus (*3*) for *T. köningi* Oudemans, and another, with spherical or nearly spherical spores, showed the absence of zonate spore-tuft production attributed to *T. köningi*. Frequent chlamydospore formation, described as characteristic of *T. köningi*, was observed in some of the strains whose isolation was not attempted. It appears that both species, if they are to be regarded as distinct species, are more or less common in pine seed beds.

In an early inoculation test with an unidentified strain of Trichoderma from Washington, D. C., an autoclaved flat was sown with jack pine, inoculated broadcast, and kept under moist-chamber conditions without result. In a later experiment (No. 31, Table II) with the elliptical-spored strain above referred to, the nonzonate strain, and the more typical *Trichoderma lignorum* strain, in cultures of uncertain purity, were used in heavy inoculation on five pots each with actually less parasitism than occurred in the controls. A fourth culture of *Trichoderma* sp. of doubtful purity resulted in somewhat increased damping-off, but within the limit of experimental error.

In the experiment on sand treated with acid and lime, referred to in the accounts of experiments with the preceding fungi, one pure and two doubtfully pure cultures representing the three different types were tested. The most typical strain of *Trichoderma lignorum* had no effect on western yellow pine and little or none on jack pine. The nonzonate form and the elliptical-spored form both appeared to decrease germination in both pines, the former also increasing damping-off in western yellow pine and the latter in jack pine. The differences were in all cases within the limits of accidental variation. The indications from these tests are that the strains of Trichoderma used are either unable to cause the usual type of damping-off or are very unimportant causes. The frequency with which the fungus is obtained in planted-plate cultures from dead seedlings may easily be due to its common presence in soil and its capacity for rapid growth on the agar used.

In addition to the usual type of damping-off, the blacktop type, mentioned in the first part of the paper and illustrated in Plate B, figures 2 and

3, is to be considered in connection with Trichoderma. Plate cultures from 30 jack-pine seedlings affected with blacktop in a nursery in the Nebraska sand hills in every case yielded species of Trichoderma; no such uniformity of occurrence has been encountered for any fungus in any of the series of cultures made from other types of damping-off.

The blacktop type occurs rarely, and, so far as observed, only under unusual weather conditions, most of that observed having followed unseasonably cold, wet weather. Extensions of the lesions into unaffected tissue stopped simultaneously and abruptly throughout the beds, apparently because a change in conditions increased resistance. The affected seedlings were scattered throughout the beds, seeming equally common on acid-treated and untreated areas. The entire picture was that of a disease caused by a fungus which is not strongly parasitic, and is well adapted for aerial dissemination. *Trichoderma* sp. seems to fulfill both of these requirements, and uniform association with the lesions is believed to indicate causal relationship. The fact that no such lesions have been produced in the few inoculations with *Trichoderma* sp. does not exclude this hypothesis, as the field evidence indicates that the lesions occur only under very unusual conditions, which are not well enough understood to be duplicated in artificial inoculations.

PESTALOZZIA SPP.

Species of Pestalozzia have occasionally appeared in cultures made by planting damped-off seedlings in prune-agar plates. In some cases the spores had two dark cells, suggesting *P. hartigii.* The occurrence of *Pestalozzia* spp. in cultures from damped-off seedlings has not been sufficiently frequent to indicate parasitism strongly. Some interest, however, attaches to the positive inoculation results reported by Spaulding (*25*). The fact that *P. funerea* is widespread and common on dead coniferous material and that he succeeded in killing 1-month-old seedlings of western yellow pine by inoculation with pure cultures of it indicates that it may be of some importance as a cause of damping-off. His single experiment is the only one which has been noted on seedlings still of damping-off age. Further experiments with it on young pine seedlings are desirable. Its slow growth and rather slow fruiting tendencies would make it rather difficult to demonstrate in diseased seedlings always filled with fast-growing saprophytes, so that failure to obtain it frequently by cultural methods is no proof that it does not occur more or less frequently in the seedlings.

BOTRYTIS CINEREA

Botrytis cinerea (*B. douglasii, B. vulgaris*), frequently connected with the damping-off of seedlings of various plants and with needle diseases of the young shoots of coniferous seedlings, does not seem to have been reported as causing damping-off of conifers. *B. cinerea* has never appeared in the

writer's cultures from Colorado and points farther east, but was easily obtained from western yellow-pine seedlings growing in sterilized soil in the plant pathological laboratory at the University of California. The lesions on which it was first obtained occurred just above the soil surface and produced the Botrytis spores directly on the lesions almost as soon as the lesions themselves became evident. The spores and sporophores appeared to be typical of *B. cinerea*.

Inoculations had been made prior to this time with a culture of *Botrytis cinerea* from apples from the Pacific Northwest furnished by Dr. J. S. Cooley, of the Bureau of Plant Industry. The pots inoculated were kept covered with glass plates from the time they were autoclaved until the close of the experiment. Jack pine was used in the test, and the inoculum was probably a mixture of cultures on steamed rice and prune agar (record on this point is lacking). Parallel tests were made on three soil types. On two of these the germination was very poor in all pots, and damping-off was present in the controls as well as in the inoculated pots. In the third soil type, a poor soil from Takoma Park, D. C., in which inoculations of *Pythium debaryanum* had proved unsuccessful, 44 seedlings appeared in the five control pots during the first few days after germination began, while in the five pots of *B. cinerea* but two seedlings appeared in the same time. Additional seedlings appeared later in both, but with still approximately three times as many in the control pots as in the pots inoculated with the fungus. Damping-off was somewhat, but very little, heavier in the Botrytis pots. The experiment indicates germination loss as a result of the presence of *B. cinerea*, but must be repeated to give conclusive results.

Mainly because of the known parasitic ability of *B. cinerea* on seedlings of other plants and on older conifers and the strong indications of parasitism seen in the seedlings at the University of California, rather than on the basis of the single inoculation experiment, it is believed that this fungus will probably be sometimes found causing damping-off of conifers. It is a fast-growing organism on prune agar, and failure to obtain it at most of the nurseries at which cultures have been made is strong evidence that it is not present in the damped-off seedlings at these places. Spaulding's failure to obtain it frequently from the seedlings with which he worked (*26*) is further evidence that in the East and Middle West at least it is not important as a cause of damping-off of conifers.

MISCELLANEOUS FUNGI

Alternaria sp. has been very frequently encountered in cultures from dead coniferous material, both in the tender seedling stage and in older nursery stock. No inoculation tests with it have been made. It is not believed to be important as a damping-off parasite, but its frequent

occurrence, especially on damped-off seedlings under moist chamber conditions, makes a test of its parasitism desirable.

The common large *Mucor* sp. is another fungus frequently found in agar cultures from damped-off pine seedlings. Two strains, one apparently pure and the other of somewhat doubtful purity, have been tested in inoculation on jack pine in autoclaved soil. In experiment 31 (Table II shows results of other fungi in this experiment) there was distinctly less damping-off in the 10 pots inoculated with cultures of *Mucor* sp. than in the 25 control pots. In the experiments on sand treated with acid and lime, the two Mucor cultures gave slight indication of parasitism on jack pine and still less indication on western yellow pine. In both cases the differences between the inoculated and control plots were well within the limits of accidental variation. It seems probable that strains of Mucor are not of any importance as damping-off parasites and that their frequent occurrence in the cultures from seedlings, as in the case of *Trichoderma* sp., can be explained by the fact that *Mucor* sp. is a common soil-inhabiting saprophyte and is able to grow very rapidly in prune agar.

Penicillium sp. of the common green type has occurred more or less frequently in cultures from damped-off seedlings. Mixed cultures containing *Penicillium* sp., like the cultures of *Mucor* sp., apparently caused a decrease rather than an increase in damping-off in the 10 pots of jack pine to which they were added in experiment 31, and on both jack and western yellow pine in the experiment on soil treated with acid and lime.

Aspergillus sp. of the common type with black spore heads, obtained from damped-off western yellow pine at Washington, D. C., when inoculated on five pots of jack pine in experiment 31, apparently caused a decrease rather than an increase in damping-off. In the experiment on jack and western yellow pine on sand treated with acid and lime, the same was true for this culture, and also in the case of western yellow pine for another strain of Aspergillus taken from damped-off western yellow pine from the Kansas nursery, at which the experiment was conducted. This latter culture had no effect on jack pine. *Aspergillus* sp. has not appeared in culture from seedlings often enough to warrant any suspicion that it is concerned in causing damping-off.

Phoma betae (Oud.) Fr. (pure culture furnished by Dr. H. A. Edson) in experiment 31, Table II, had no apparent effect on damping-off of pine. The same held true in western yellow pine in the experiment on sand treated with acid and lime, and while a slight decrease in germination and a slight increase in damping-off was noted in the jack pine inoculated with *P. betae* in this latter experiment, the difference was easily within the limit of accidental variation. The failure of this damping-off parasite of beets to affect pines was to be expected, in view of the specialization of the fungus to beets indicated by the systemic character of the infection it produces on them (*8*).

Two pots of pine in autoclaved soil were heavily inoculated with agar cultures of the species of Phoma which has been shown to cause the blight of young cedar (*10*). No damping-off resulted, although in the same experiment both *Corticium vagum* and *Pythium debaryanum* proved strongly parasitic. It has not been found in cultures from damped-off seedlings.

An unidentified species of Phoma from blighted 2-year-old seedlings of western yellow pine from Montana, used on five pots of jack pine in experiment 31, apparently resulted in decreased rather than increased damping-off.

Inoculation with a bacillus which appeared commonly with *Pythium debaryanum* from damped-off seedlings also appeared to decrease rather than increase damping-off in experiment 31, and in the experiments on soil treated with acid and lime to increase the loss, but in both cases to an extent explainable as accidental variation.

The statement made in several of the foregoing paragraphs that the organisms considered apparently decreased damping-off in experiment 31 is not necessarily paradoxical, in view of the possibility of competition between saprophytic organisms and accidentally introduced parasites.

It is realized that not all of the possible damping-off parasites have been tested. In some cases it has proved very difficult to obtain from damped-off seedlings any of the known parasitic organisms. It is desirable to make further attempts to obtain, and to carry on inoculation experiments with, slow-growing organisms not likely to appear in the planted plate cultures used by the writers for isolation purposes.

OTHER PARASITIC DISEASES

Discosia pini Heald has been found on western yellow pine in the same way as described by Heald (*17*) and at the same nursery. Observations confirm his conclusion that it does little or no harm. Cultures were obtained by making dilution plates of the spores, but the subcultures were not carried long enough to obtain fruits. The same fungus was found occurring in just the same way on white pine from a nursery in Georgia.

Another fungus occurring on living pine seedlings of the damping-off age without causing the decay typical of damping-off is the European rust *Melampsora pinitorqua* (A. de B.) Rostrup. This rather dangerous parasite has not so far been reported authentically from America, and its importation should be very carefully guarded against. It most commonly attacks the young needles and shoots of trees 10 to 30 years old, but it may also produce its orange sori on seedlings which have just appeared above the soil surface (*11*, *24*). Hartig (*11*) has found it attacking as many as two-thirds of the seedlings in a stand of Scotch pine, producing spores on the cotyledons and hypocotyls when the seedlings were only 2 months old. Seedlings recovered whose cotyledons

only were attacked. It is reported on Austrian and Corsican pine, on *Pinus montana*, and even on white pine (*5*). The same or a similar rust has been reported on species of Abies (*24*). The alternate stage occurs on poplars (*Populus tremula*, *P. alba*, *P. balsamifera candicans*, etc.), and as it attacks stems as well as leaves, importation of poplar as well as of coniferous nursery stock may introduce the disease.

WHITESPOT

Light-colored shrunken lesions sometimes appear at the bases of the stems of pine seedlings as a result of excessive heat at the soil surface. The lesions (Pl. B, fig. 4, 5) are characterized by shrinkage, light color, definite limitation, and the fact that such one-sided lesions as occur are usually on the south side of the stem. They have undoubtedly been confused with damping-off in the past. Unlike damping-off, the lesions do not extend longitudinally until some days after their appearance and then apparently only as a result of the invasion of the original lesion by fungi. Upward conduction of water is not interfered with. Losses are occasionally serious, especially where there is little shade or the soil is loose and inclined to become dry at the surface. This type of injury has been described in more detail by Münch (*19*, *20*) and Hartley (*15*), who present evidence as to its relation to heat. A case of damping-off recently reported in red pine (*18*) in which the loss was limited to soil containing raw humus allowed to dry at the surface may be an example of confusion between whitespot and parasitic damping-off. Münch emphasizes the likelihood of heat injury on raw humus soils.

MECHANICAL INJURIES

Mechanical injuries of different types are rather frequent in coniferous seed beds during the first month after germination. This is particularly true of species which have small seed and therefore produce delicate seedlings. Red pine, with a seed of intermediate size, is also very subject to mechanical breakage because of the unusual brittleness of the stems during the first two or three weeks after the seedlings appear above soil. Counts of obviously mechanically injured seedlings were continued throughout the damping-off season in beds of red, jack, and western yellow pine in Nebraska, Kansas, and the Lake States, in connection with seed-bed disinfection tests. A survey of the data on a large number of the untreated plots taken at random shows that the total loss from mechanical causes may vary from 1 to 10 per cent of the entire stand, with losses of 3 to 3.5 per cent common, and below 2 per cent or over 5 per cent rare.

Types of mechanical injury which cause loss in the seed beds are (*1*) the washing out of the germinating seed or of very young seedlings; (2) the actual breakage of the stems by high wind, hail, beating rain, or the feet of birds or animals; (3) the eating or pulling up of the seedlings

by insects, rodents, or birds; (4) the cutting off of the roots below ground by insects in the soil which presumably feed on them, or by moles which accidentally break them in working through the soil; and (5) the breaking over of the stem, usually at the soil surface, as a result of repeated bending by wind or possibly by other agencies, sufficient to finally cause the collapse of the cortex without transverse rupture of the epidermis or vascular system.

The first four types need little mention, as there is no danger of their being confused with any of the other types of disease mentioned in this article. A few observations on the factors influencing the washing out or breakage of seedlings in artificial watering may be of interest. It develops that the fine spray from nozzles which some nurserymen use in watering germinating seed beds will in some cases cause much more loss through the washing out or breakage of seedlings than some other methods of watering. The best types of stationary sprinklers are less harmful. Another much more drastic method of applying water has also been found relatively harmless. It appears that the washing out which commonly occurs is due not so much to the amount of water applied as to the angle at which it strikes the bed. This at least seems to be the case in the sandy soil where most of the writers' watering experiments were conducted. A fairly strong spray from the nozzle, by striking the surface of the bed at an angle, as it practically always does, seems to displace soil particles to a much greater extent than a very much heavier mass of water striking the surface of the bed directly from above. Very shallow-sown seed beds have been watered without injury, by applying the water directly from the end of a 1-inch hose line without nozzle or even coupling, the end of the hose being so held that the water was spread out over the hand of the man holding it and fell vertically on the beds in a rather thin sheet. In this way water was applied to the beds with good pressure from large mains and hose leads at the rate of 15 gallons per minute, while on the same beds a nozzle closed so as to give a spray and delivering only from 3 to 5 gallons per minute caused considerable washing out.

The fifth type of mechanical injury mentioned in the foregoing is one which, though probably not uncommon, is very difficult to distinguish from whitespot due to heat, and may also be confused with damping-off. Plate B, figure 7, shows a seedling which was found broken over with characteristic white-spot symptoms at a time following high wind and cold, cloudy weather. The conditions were such as to seem to preclude the possibility of heat as the cause, and the lesion differed from the ordinary heat lesion in having the constriction more definitely limited to a particular point, not involving the stem either above or below that point. Much the same symptoms were later produced in a seedling of western yellow pine by exposing it to a strong wind. Simple mechanical bending was then tested on seedlings of jack, red, and western yellow pine. As expected from the experience in the seed beds, lesions such as those in

figure 7 could not be readily produced by bending red pine, as the stems broke squarely off, and immediately wilted. In one or two cases, however, breaking over with one-sided lesions was obtained without apparent rupture of the epidermis. In western yellow pine repeated bending, not carried quite far enough to break the stem, in most cases produced a collapse of the cortex at a point on the side away from the application of the bending force, with a resultant weakening of the stem at this point so that a sharp right-angled bend could be made without further injury to the stem. In long repeated bending the entire perimeter became more or less constricted at this point and the seedling lost its ability to stand erect. With jack pine, the species in which the supposed wind injury was observed in the seed beds, similar lesions were more easily produced either by artificial bending or by the application of a strong draft of air from a pressure vent. If the mechanical bending included a slight twisting movement or if the seedling was so placed that it was whipped sideways as well as downward by the blast of air directed against it, the collapse of the cortex at the point of bending could be made even more complete than that observed in the nurseries. The whitening of the stem both above and below the constriction, shown in Plate B, figure 7, was not produced by artificial bending or exposure to air currents to any great extent except in lesions which occurred just at the soil surface. It is believed that the change of the epidermis above and below the point of constriction from red to nearly white, indicating the loss of sap from the cells, came as a result of bruising against the soil rather than as a result of bending alone. The stems of young seedlings are so susceptible to bruising that even stroking the side of the stem with a smooth piece of wood or a rubber-tipped rod results in the development of a lesion characterized by sunken surface and more or less discoloration.

What apparently takes place in these cases is the loss of sap from the cortical tissue, and probably, in view of the loss of red pigment, from the epidermal cells as well. Whether this is a result of an actual rupture of the internal tissues or from an increase in the permeability of the tissue as a result of the mechanical stresses developed is not known. It should be pointed out that there is nothing especially different from the collapse of the cortex under bending and collapse due to external pressure. In both cases the injury is believed to be caused by pressure. This belief is supported by the fact that in the case of experimental bending it is the cells on the side of the stem on the inside of the bend which first collapse, these, of course, being the ones which get the most pressure in the course of the bending. That the vascular tissues are not seriously affected is evidenced by the continued turgor of the leaves. In a seedling with a much more serious and extensive lesion than the one figured, resulting from mechanical bending, the leaves were still turgid 16 days after the injury, and the prostrate stem had shown negative geotropic response,

the cotyledons and the upper part of the stem turning upward away from the soil. The epidermis, while showing no evidence of serious injury beyond the loss of pigment, may suffer microscopic ruptures. On the upper side of a yellow-pine seedling at the point where a lesion was being developed by bending, observation with a hand lens showed that minute drops of liquid were extruded from the epidermis apparently at small ruptures, which, however, could not be detected after the drops had disappeared. Stomata may, of course, have been the points of exit of the sap.

The importance of lesions produced by bending without outright breakage in the seed beds is slight, unless very high winds occur during the first 10 days after the appearance of the seedlings. The fact that the lesions, like white-spot lesions due to heat, remain definitely limited for a week or more, together with their lighter color, serves to distinguish them from those caused by the usual damping-off organisms. Positive differentiation on the basis of color alone is not entirely safe, three or four days' observation being necessary to determine whether or not a lesion is parasitic.

It may be mentioned that in older plants a girdling of Russian wild olive, several months old, by a transverse lesion at the soil surface, followed by death a number of weeks later, and the girdling and local collapse of the soft young shoots of 2- or 3-year-old pine nursery stock, both observed in regions of high wind, may also prove to be due to excessive bending.

DROUTH INJURY

Death of young seedlings from drouth is undoubtedly sometimes confused with damping-off. Seedlings so young that the stems have not finished elongating may remain erect for some time after being killed either by drouth or normal damping-off, as the compactness of the tissues apparently prevents immediate collapse from water loss, and decay takes place but slowly. When the seedlings are old enough to have stiff, wiry stems, difficulty again arises in distinguishing between seedlings killed by drouth and those killed by the late type of damping-off. Therefore during the first week and after the third week from germination it is not usually possible to distinguish drouth injury from damping-off by the condition of the individual seedling. Fortunately the distribution of the diseased seedlings often gives a clue. Damped-off seedlings, especially in the late damping-off type, are usually so grouped as to definitely indicate infection foci. This is not the case in drouth injury.

At the intermediate age of one to three weeks it is often possible to distinguish between drouth injury and damping-off by the condition of affected seedlings. At this stage seedlings dying from drouth show prompt and unmistakable wilting, while the damped-off seedling at first remains turgid, typically falling on account of the decay of the base of the stem before wilting of the rest of the stem takes place. Seedlings just

fallen over as a result of water shortage therefore exhibit uniform bending of the entire stem, while seedlings fallen from damping-off or from white-spot usually have a rather sharp bend in the lower part of the stem, and little or none in the upper part.

In a number of western commercial nurseries in which no provision is made for artificial watering drouth injury is in certain seasons a serious matter. During a normal season and in a loamy soil loss from drouth in first-year seed beds is probably not a serious matter east of the Mississippi River. In nurseries, first-year seedlings more than a month old apparently suffer less often from drouth than 2- or 3-year-old seedlings as they have their roots well established, but are not large enough to crowd each other and rapidly exhaust the soil moisture.

Soft-stemmed seedlings so far injured by drouth as to be badly wilted may still recover if supplied with water within a few hours after wilting.

SUMMARY

(1) Damping-off is the most serious disease of very young seedling conifers. A number of symptomatic types are described, part of which are figured in Plate B.

(2) *Corticium vagum*, *Pythium debaryanum*, and other oomycetes; *Fusarium moniliforme*, *F. ventricosum*, *F. solani*, and other species of Fusarium; *Trichoderma* spp.; and *Botrytis cinerea* have been isolated from damped-off conifers, and are believed to be able to cause the disease. Spaulding's work indicates that *Pestalozzia funerea* can also cause damping-off of pine. In artificial inoculations on pines in autoclaved soil, the first three species named proved to be especially virulent parasites. All except *Trichoderma* spp. have given more or less indication of parasitic ability in inoculation experiments.

(3) For *Corticium vagum* 12 coniferous hosts are listed (p. 531). One strain was maintained in artificial culture continuously for eight years without perceptible loss of virulence. There is a marked difference in virulence between different strains, which bears little or no relation to the host from which the strain was isolated. For example, strains from spruce and sugar beet, respectively, proved more virulent in inoculations on pine seedlings than did any of the strains originally isolated from pine. There was furthermore no indication that passage through seedlings and reisolation resulted in any increase in virulence. *C. vagum* was found especially virulent in inoculations on a very sandy soil treated with sulphuric acid, followed by lime. Slight virulence was indicated in preliminary inoculations on untreated soils.

(4) With the possible exception of *Pythium debaryanum*, *Corticium vagum* appears to be the most important single damping-off parasite on conifers, certain species of Fusarium also being probably important, and the remainder of the organisms mentioned unimportant. The available

data do not justify a final statement on the relative importance of the different parasites.

(5) The following fungi have indicated inability to cause damping-off of pines: *Aspergillus* sp. (the ordinary black type); *Penicillium* sp. (the ordinary green type); a rapidly growing species of Mucor from damped-off seedlings and seed-bed soil; *Trichothecium roseum; Fusarium acuminatum; Rosellinia* sp. from nursery soil; *Chaetomium* sp. from maple roots; *Phoma betae;* the species of Phoma which causes red-cedar blight; and a third species of Phoma from pine in Montana.

(6) At least with some of the fungi found parasitic, heavy inoculation and heated soil so favor parasitism that past experiments, mostly conducted under such conditions, do not constitute an entirely reliable basis for deciding what goes on in the seed beds under more natural conditions.

(7) Inoculations in autoclaved soil with certain saprophytic or weakly parasitic fungi have apparently resulted in a decrease rather than an increase in damping-off in some cases.

(8) *Corticium vagum* and *Pythium debaryanum* cause a large part of the damage they do by killing seed or seedlings before they appear above soil. Strains of Fusarium are less inclined to do this. Such losses are often wrongly attributed to poor seed. Some of the damping-off fungi are able to continue to kill the roots of seedlings after they develop rigid stems, so that they do not fall over. This type of trouble is sometimes confused with drouth.

(9) Excessive heat, drouth, or bending may each cause injury closely simulating damping-off. On careful examination these nonparasitic types of injury can usually be distinguished from damping-off by characters described in the last pages of this paper.

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PLATE B

1. Normal damping-off on western yellow pine; caused usually by *Corticium vagum*, *Fusarium* spp., or *Pythium debaryanum*. Natural size.

2, 3. Blacktop damping-off on jack pine; probably caused by *Trichoderma* sp. × 2. —— = location of surface of soil.

4. Whitespot injury, common type, on western yellow pine; usually due to excessive heat at soil surface. Natural size.

5. Whitespot lesion, one-sided type, on western yellow pine; due to heat. Natural size.

6. Late damping-off resulting from inoculation with *Pythium debaryanum* on red pine more than 5 weeks old. × 1½. —— = location of surface of soil.

7. Wind injury to jack pine. This seedling had fallen over and was propped up for drawing purposes. ×2.

Drawn by Maybell S. Hartley.

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HISTOLOGICAL STUDIES ON POTATO LEAFROLL[1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE AGRICULTURAL EXPERIMENT STATION AT CORNELL UNIVERSITY

INTRODUCTION

External symptoms are often insufficient and sometimes misleading in the identification of certain plant diseases, and the pathologist must resort to the studies of internal morphology and physiological reaction to bring otherwise problematical questions to a satisfactory conclusion.

The necessity of such an attitude is most clearly exemplified in the case of the Irish potato leafroll diseases, which have been the subject of much discussion and controversy for the last decade, both in this country and in Europe.

The literature of potato leafroll is voluminous and varied. The most important articles, about 600, were reviewed by Appel and Schlumm-berger (*1*)[2] in 1911, and about three years later this list was extended by Orton (*4*), in which he reviews the history of this disease and related troubles and the theories regarding its cause.

The ready identification of leafroll in the field and its separation from other maladies is difficult, and there is much confusion over diagnosis. Our knowledge of the pathological physiology of this disease is incomplete, and an understanding of the histological changes in the diseased tissues is only in its beginnings. Very little is known in regard to the chemical changes which accompany the disorganization processes in the cells and tissues, and nothing definite is known about the origin and progress of the disorganization which sometimes leads to a complete obliteration of the walls and contents of the elements of the conducting system for plastic materials.

The pioneer researches of Quanjer (*5*) have given an impetus to new investigations along the lines just mentioned and have diverted our views from the fungus culture to the histological and physiological side of the problem.

[1] This work was begun in the field laboratory of the Office of Cotton, Truck, and Forage Crop Disease Investigations at Greeley, Colo., in the summer of 1916, and was continued in the Department of Plant Pathology at Cornell University under the direction of Prof. H. H. Whetzel and Dr. H. A. Edson, to whom the writer wishes to express his gratitude for their courtesy and helpful suggestions. To Dr. A. J. Eames, of the Department of Botany at Cornell University, the writer is especially indebted for the constant advice and criticism received in preparation of materials, interpretation of slides, and editing of the paper.

[2] Reference is made by number (italic) to "Literature cited," p. 570.

While taxonomic, inheritance, and control studies are being carried on at present by various investigators, the following studies have for their purpose the testing of Quanjer's hypothesis with both European and American material, and the adding of new facts to the already existing knowledge. A former paper (2) on the anatomy and development of the normal Irish potato plant furnished a background for the pathological study and facilitates a more accurate interpretation of the existing conditions.

It is realized that an adequate inquiry into the problem of potato leafroll from the viewpoint of the morphologist should first of all comprise a detailed seasonal study of the normal plant grown under different ecological conditions, with the purpose of correlating the influence of external factors with changes in internal anatomy. These investigations should then be extended to a study of plants affected with diseases of parasitic and physiologic origin, to determine in how far the delicate internal structures respond to the stimulating effect of parasites and their toxins, as well as to unfavorable conditions of growth.

A detailed microscopical study extending over several generations should make it possible to trace the development of the disease from the first cell response through the various changes, culminating in the cessation of function and death; while microchemical investigations should afford an insight into the physiological activities of the diseased plants, especially in respect to assimilation and translocation. A critical study of the data obtained should either emphasize or discredit the value of comparative anatomical studies for diagnostic purposes, and might permit a grouping or separation of diseases on the basis of pathological anatomy according as the symptoms found were similar or unlike.

Present circumstances make it necessary for the writer to interrupt studies of this character for an indefinite period while they are still incomplete and preliminary, but sufficient progress has been made on a few of the general questions of foremost interest to justify their presentation in the hope that they may be of interest and value to other investigators. This paper will be limited to the presentation of a comparative study of certain European and American types of leafroll, together with observations on the progressive histological changes in the tissues.

EXPERIMENTAL MATERIALS AND METHODS

Tubers for the study of European leafroll were obtained from Dr. Quanjer, Wageningen, Holland, and from the Agricultural College, Copenhagen, Denmark. The material representing American leafroll was obtained from the Government station in Greeley, Colo., and from various places in the State of New York.

The methods of killing, fixing, embedding, and staining the material were the same as those used (2) for the normal Irish potato (*Solanum tuberosum*).

DESCRIPTION OF EUROPEAN LEAFROLL

LEAFROLL IN PAUL KRUGER VARIETY

Tubers from both normal and diseased potato plants were grown in the disease garden of the Department of Plant Pathology of Cornell University. The plants from the diseased tubers grow more slowly than the normal ones, show a shortening of the internodes of the stem (Pl. 35, A, B) and a pronounced development of axillary shoots later in the season (Pl. 37). The lower leaves, and later the upper ones, become rigid, and the leaflets fold more or less along the midrib. The whole leaf shows the effect of the rolling, and exhibits a spiral tendency, causing the mature plants to stand out prominently among normal individuals. The color of the foliage changes with the progress of the other symptoms. At first the lower leaves show a pale discoloration; in the beginning this is confined to the tip and the margin of the leaflets, gradually spreading and changing to a reddish tinge. At the time of maturity of the plant the tissue of many of the discolored areas is dead and of a brown color. The tubers set normally, but on rather short stolons; the yield is small both as regards number and size. The seed piece is found unexhausted in the soil.

Cross sections through midrib and lamina of a leaf of such a plant show, on the whole, normal histological structures. The fibers, however, are more plentiful, and high magnification reveals slight changes in the peripheral region of the vascular tissue. Here and there a few cells of the pericycle are found to be filled with a deposit, sometimes crystalline, sometimes of globular masses, yellow in unstained sections. When stained with Haidenhein's hematoxylin and safranin, the deposit stains reddish or remains yellow. Mineral acids, either dilute or concentrated, do not dissolve this precipitate; alkalies increase the intensity of the discoloration. Treatment with phloroglucin and hydrochloric acid to test for lignification gives negative results; alcanin, however, produces a reddish color suggesting the presence of cutin or cutin-like substances (Sudan III and Scharlach R, the most typical reagents for cutin are unavailable at present). In other places intercellular spaces have formed between the cells of the phloem. These cavities are filled with a secretion rather plastic in nature and reacting similarly to the treatment of chemicals as does the crystalline deposit found inside the cells just mentioned. The cells of the pericycle in such a region are radially elongated and slightly distorted.

Sections through the lamina show a disorganized condition both of palisade tissue and of spongy parenchyma in those regions which show external symptoms of the disease most strikingly. The cell wall, though not thickened, has undergone chemical changes, having become cutinized. The lumina of these cells are filled with a precipitate of the nature described for the diseased cells of the vascular tissue of the midrib. The

petiole, like the midrib, also shows normal conditions as regards the amount and arrangement of vascular tissue. The fibers are more numerous and of a larger diameter than those found in normal sections. The secondary walls of these elements are often very thin and only very slightly lignified. The smaller type of fiber, especially numerous in the inner phloem, has its secondary walls heavily lignified, and the lumina are sometimes filled with the granular deposit. Intercellular secretions are evident in the peripheral region of the outer phloem and in some of the cells of the pericycle. The inner phloem is, on the whole, normal. Plate 39, A, shows a disorganized phloem group with the wheel-shaped arrangement of the parenchyma cells surrounding it.

The diseased condition becomes more pronounced in the growing region and especially in the nodal region of the upper part of the stem. Cross sections through the upper three internodes show nearly all types of pathological changes observed in this study. Plate 38, C, and Plate C, figures 1 and 6, illustrate the formation of intercellular cavities between the sieve tubes of an inner phloem group. The primary wall separates—a fact not easily observed in unstained material—and the space resulting from the separation of these cells is filled with a yellow substance, gummy and plastic in nature. Similar changes occur in the outer phloem (Pl. 38, B, and Pl. C, fig. 2). In more advanced stages the formation of intercellular spaces has progressed to the extent of causing bending in and crushing of the phloem cells. In the final stage of disorganization the phloem cells are almost always filled with a granular substance, while the walls themselves have become cutinized or undergone similar changes (Pl. C, fig. 4). Lignification of either cell wall or cell content has never been observed.

The diseased condition is most severe in the nodal region of the upper stem, and it is mostly the outer phloem that is affected. However, cells of tissues other than the phloem exhibit pathological changes. Plate 38, A, and Plate C, figure 3, show the effect of the disease in the cells of the cortex and pericycle. The large cortical cells adjacent to the fibers are filled with the granular precipitate, the walls themselves having become cutinized, and the small triangular intercellular spaces are filled with the yellow secretion. Intercellular spaces are seen to be forming between the cells of the pericycle; they originate among the outer fibers and gradually advance toward the cambium. The cells of the pericycle have become radially stretched and at the same time have increased in number, forming a broad sheet of tissue between the fibers and the primary phloem (Pl. 42, A). In normal sections the primary phloem groups, if not directly adjacent to the fibers, are almost always separated by only one or two layers of cells.

A different type of pathological condition is sometimes observed in both stem and leaf sections. Plate 39, B, shows a section taken from the internodal region of the middle part of the stem. Only the cells of the

inner phloem are affected. The individual groups are normal in size; there are a few cell wall thickenings, but no evidence of formation of intercellular spaces. Most of the walls of the sieve tubes and companion cells have become faint and in places are completely obliterated. The cell content is mostly disorganized protoplasm. These diseased groups take the cellulose stain, less distinct, however, than the normal cells.

The basal region of the stem and the underground parts are normal in structure; the flowering axis has not been examined.

LEAFROLL IN MAGNUM BONUM VARIETY

The tubers were obtained from the Agricultural College, Copenhagen, Denmark, and planted, together with other varieties under investigation, in the disease garden of the Plant Pathology Department of Cornell University. The plants grew normally at first; most of them, however, developed signs of disease in late summer, though a few showed symptoms of leafroll very early. Material for study was taken in late July from a plant which was large and well developed, but the axillary shoots of which were erect and broomlike in appearance. The lower leaves were normal, the intermediate and upper ones reduced in size and folded along the midrib. Discoloration of the leaves was only slight.

Microscopic examination of both stained and fresh material shows that the vascular tissue of the midrib is abnormal in quantity with thickenings of the walls of the phloem adjacent the fibers (Pl. 45, A). These thickenings stain black with Haidenhein's hematoxylin—that is, they are of cellulose. The lamina of the leaf, however, is severely diseased. The partly obliterated cells are filled with a granular or globular deposit, which is yellow in unstained sections and of the same nature as found in the diseased cells of the phloem in the Paul Kruger variety. The petiole shows also normal anatomical structures. The bundles of the petiolar wings are above normal in size. Fibers in both external and internal region are of unusually large number (Pl. 45, B).

Stem sections, whether taken near the distal or the basal end, show normal structures. The fibers here too are very plentiful. The external phloem shows a large amount of secondary sieve tubes and medullary ray tissue, all of which appears normal and functional.

LEAFROLL IN EARLY ROSE VARIETY

The tubers were obtained from the same source as were those of the Magnum Bonum variety; they were planted at the same time and received the same cultivation and care. The plants grew very unevenly. A few of them attained normal size, but most of them remained small, showing symptoms of severe leafroll. Plate 36 shows such a plant. The stem is not above 15 cm. tall; the leaves are rolling, but are not reduced in size. There is slight, indistinct discoloration of the lamina.

The general amount and arrangement of the vascular tissue of the midrib is normal. Fibers are wanting, and the phloem groups of the inner

region show disorganization of cell content and partial obliteration of the walls of the individual cells. The spongy parenchyma of the lamina is abnormal in places; the cells in such a region are filled with a yellow deposit and the walls themselves are of a yellow color. The petiole is normally developed. Fibers are present, and the phloem cells in the region of the inner fibers are more or less thickened.

Stem sections are, in general, normal. While the outer phloem shows no evidence of pathological condition, the cell walls of the inner phloem groups are partly destroyed and the protoplast disorganized. In no instance, however, is there a thickening or discoloration of the walls of these cells.

A summary of the results of microscopic study on European leafroll shows the following conditions:

1. Plants with symptoms of typical leafroll always show pathological changes in the vascular tissue.

2. These pathological changes are the more pronounced the earlier and more intense the external symptoms appear.

3. While the primary phloem groups are most commonly affected, the cells of the pericycle and fibers as well as cortex are often found diseased.

4. The phloem groups are shrunken and completely destroyed only in rare cases. Most usually the cells of the groups are separated by intercellular spaces; the cells may later be crushed and the cell content changed into a granular or globular, gummy deposit. There is seldom a complete destruction of the individual cells in a group and never a sign of lignification.

5. The number of primary phloem groups destroyed is not always very extensive, and since the development of secondary elements is very pronounced the translocation of elaborated food material from the leaves to the tubers is not prevented or only slightly interfered with.

DESCRIPTION OF AMERICAN LEAFROLL ON COLORADO MATERIAL

The cause of the falling off in yield of the Irish-potato crop in northern Colorado in 1911 was thought by local growers to be species of Fusarium and Rhizoctonia, but others have believed that, though there is a variation in the external symptoms observed, the Colorado disease is the type of leafroll described by Appel and other investigators in Europe.

The material for study was obtained from the Government station at Greeley, Colo., and included one seedling and two standard varieties: Netted Gem and Pearl. These plants were under observation throughout the summer, the progress of the symptoms being recorded at definite intervals.

LEAFROLL IN POTATO SEEDLING

The mature plant was dwarfed, erect and broomlike in appearance. The leaves were reduced in size and folded characteristically along the midrib. The seed piece was found unexhausted in the soil.

Cross sections through the petiole show normal structures. A few cells of the inner phloem have walls thickened adjacent to the fibers. The cells of the epidermis, though normal in structure, are filled with a dirty-gray granular precipitate. The stem tip, especially in the nodal region, shows a few shrunken inner phloem groups. The cells of the pericycle are slightly stretched radially; a few of the cells have become separated and the intercellular spaces formed are filled with the yellow secretion. Other cells in the same region, but including the cortex, are filled with the granular precipitate (Pl. 41, B) characteristic of the diseased tissue in the plants of the Paul Kruger variety (Pl. 38, A). The symptoms become less pronounced lower down the stem; yet here and there completely disorganized phloem groups are seen, especially in the inner region.

EFFECT OF LEAFROLL NO. 15000 ON AN UNKNOWN VARIETY

This variety had been selected the previous year (1915) for suspected leafroll. The plants developed normally and attained a large size, becoming spreading later in the season. Symptoms suggestive of leafroll appeared only late in the season. The leaves rolled in a funnel-shaped fashion and showed a slight discoloration of the lamina.

A microscopic examination showed only slight pathological changes in the distal region of the stem. A few intercellular spaces were noticed between the cells of the outer phloem, and these cavities were filled with the typical yellow excretion.

LEAFROLL OF THE NETTED GEM VARIETY

At least 40 per cent of the plants grown in one of the experimental plots of the plant-breeding station at Greeley showed a characteristic abnormal rolling of the leaves, and from these plants tubers were selected in the fall of 1916 to be grown the following winter in the greenhouse of the Plant Pathology Department of Cornell University. The plants developed normally at first; later the leaves turned yellow and became dry in texture. Nearly all of the leaves showed symptoms of rolling, but unlike that observed in typical leafroll.

Cross sections through petiole show pathological changes in outer phloem and pericycle. In a few places the cells have become completely obliterated, resulting in the formation of extensive cavities. Other disease cells are filled with the granular precipitate (Pl. 41, A), in which are sometimes found embedded starch grains. Sections through the median portion of the stem show all gradations of the disease. Large intercellular spaces are found in the pericycle, and these often extend to the cambium. The fibers of the outer phloem frequently contain the granular deposit also.

A summary of the results of the study of types of leafroll from Colorado shows the following conditions:

1. The pathological changes, though less intense, are of the nature observed in the European disease.

2. Plants developing external symptoms of disease late in the season show normal histological structure of the vascular system.

3. The internal changes were most pronounced in the Netted Gem variety. Although the tubers came from stock having the appearance of leafroll, the plants grown from them did not exhibit external symptoms of typical leafroll.

DESCRIPTION OF AMERICAN LEAFROLL ON NEW YORK MATERIAL

The material was collected by the writer in the fall of 1917 on the occasion of an inspection trip through Ontario County, N. Y. The disease was quite prevalent; in a few instances as many as 30 per cent of the plants were found to be diseased. Only mature plants which exhibited characteristic symptoms of leafroll were selected for study. The material was put into fixing fluid at once and later dehydrated and embedded in the laboratory in the usual way.

LEAFROLL IN THE NEW YORK RURAL VARIETY

The material was collected in a field at Phelps, N. Y. The plant selected was fairly large, erect, and bushy in appearance. The leaves were slightly reduced in size and showed extreme rolling and reddish discoloration along the margins of the leaflets.

A microscopic examination showed, on the whole, normal development of the vascular tissue of midrib and petiole. In the outer phloem of the midrib a few abnormalities in the nature of intercellular spaces and stretching of the cells of the pericycle were found. In the petiole there were diseased areas in cortex and pith; the affected cells were filled with the granular deposit.

Sections through the growing region of the stem showed pathological changes of great intensity. Just below the insertion of the leaf in that region large lysigenous cavities have formed which extend from the cortex through the vascular ring into the pith. Longitudinally these diseased areas extend into the petiole of the leaf as well as into the upper part of the stem (Pl. 42, B, and 43, B). The peripheral phloem cells show thickenings of the walls; these thickenings are cellulose in nature. A few of the cells of the outer phloem are filled with the granular precipitate.

Median stem sections show a well-developed, normal external phloem; however the internal phloem groups are almost completely destroyed (Pl. 44, A, B). In unstained sections these diseased groups appear as small, yellow, shrunken masses from which the parenchyma cells of the perimedullary zone radiate in a typical manner. High magnification shows that these groups are either the remains of shrunken and diseased primary phloem elements, large intercellular spaces filled with the

yellow secretion, or a combination of these. Plate C, figure 5, shows such a group. We notice that most of the cells of the phloem are more or less collapsed and filled with a granular precipitate; between other cells in the group intercellular cavities have been formed, the cells themselves having remained normal.

The basal region of the stem is normal, with an occasional disturbance in the inner phloem.

LEAFROLL IN THE DOOLEY VARIETY, MATERIAL A

The material was obtained from a field near Seneca Castle, Ontario County. The plant selected for study was dwarfed, spindly, erect, and broomlike. The leaves were slightly reduced in size and folded along the midrib. Discoloration of the foliage is only noticeable along the margin of the leaflets.

Sections through midrib show normal structures; a few of the groups of the inner phloem have cells with walls and content disorganized. The petiole is severely diseased (Pl. 43, A) in that almost all of the external and most of the internal phloem is necrotic. The vascular tissue of the petiolar wings is abnormally developed; the large amount of phloem in these groups is probably formed as a consequence of the destruction of the phloem of the lateral and median bundles. Sections through the basal region of the petiole show a decrease in the severity of the symptoms; there are a few diseased areas, especially in the outer region extending from the fibers in the peripheral and radial direction (Pl. 40, A, B).

Upper stem sections show the symptoms described for the basal part of the petiole, perhaps less extreme. There are a few phloem groups completely destroyed; and in such a region the cells of the pericycle are always radially stretched. A few cells of the cortex are also diseased and filled with the typical granular deposit.

LEAFROLL IN THE DOOLEY VARIETY, MATERIAL B

Material B was obtained in the same field. The plants showed symptoms of severe leafroll; reduction in size, broomlike appearance, rolling, and pronounced reddish discoloration of the foliage.

Microscopic sections through the upper nodal region (midrib and petiole were not examined) show normal structures except for one large diseased area which extends from the cortex to the xylem, involving some of the elements of the latter tissue. Other stem sections through both nodal and internodal region show normal structures.

LEAFROLL IN THE NEW YORK RURAL VARIETY

This material was obtained from another field in Ontario County. The plants exhibited the symptoms described for the above variety, but microscopic examinations failed to show extensive abnormalities. A few stem sections showed diseased cells in the cortex which were filled with the granular precipitate.

A summary of the results of the study of types of New York State leafroll shows the following conditions:

1. There is not always a definite correlation between external symptoms and internal changes. Typical leafroll plants may not always exhibit pathological changes in the tissues.

2. The type of necrosis is that described for European leafroll.

3. In case of severe attack, American varieties showed a more striking pathological condition than did the European varieties under observation.

CONCLUSIONS

The anatomical studies of both European and American leafroll have failed so far to show a distinct correlation with the external symptoms exhibited by the plant. It is true that in case of severe attack, recognizable by external symptoms as such, we get the same pathological condition; but there are exceptions which do not permit a wide generalization. Typical leafroll plants which early show external symptoms often fail to show extensive necrotic conditions, while plants affected with trouble apparently other than leafroll, exhibit severe pathological changes in phloem and cortex. The writer, however, is fully aware that only one generation of plants has been studied and consequently he is not in a position to give full assurance that he was dealing always with hereditary leafroll. Should a study of the progeny of these plants establish the heredity nature of the disease as well as the constancy in the symptoms, it might then be possible to separate or unite the various types of leafroll on the basis of these symptoms.

There is, however, some reason to suspect that the development of necrotic tissues is not confined to plants affected with leafroll, but that it is common to the so-called degeneration troubles in general, and perhaps to others also.

The pathological changes are most striking in the distal, growing region of the stem. In sections taken farther down and especially at the basal region, signs of pathological changes are less frequent and often not found at all. An acropetal advance of internal symptoms, as claimed by Quanjer (6) for "secondary diseased" plants, has not been observed in this study. Similar observations have been made by Schander and Tiesenhausen (7) who report the earliest and most frequent occurrence of phloem necrosis in the upper part of the stem and especially in the region just below the insertion of the leaves.

The primary phloem groups are probably the first to suffer the effects of the disease. The first symptoms, as far as this study is able to show, consist of the separation of the primary walls of the phloem cells of an individual group, resulting in the formation of intercellular spaces which are filled with a secretion which is usually yellow in unstained sections. In advanced stages the primary wall of the cells, bordering these cavities, is also discolored. The size and extent of these spaces may increase to

such a degree as to cause a collapse and folding in of the walls of the phloem cells. Often, it appears, the formation of intercellular spaces, though initial, is only an accompanying phenomenon; the pathological changes in the cells of the phloem themselves proceeding so rapidly as to cause a rapid cessation in function and final death. The diseased cells become filled with a granular or globular deposit, probably metamorphosed protoplasm; the cell walls themselves also undergo changes. The deposit is resistant to acids, becomes bright yellow on treatment with alkalis, and gives reaction for cutin; in no instance, however, could the presence of lignin be shown, a fact already claimed by Schander and Tiesenhausen (7), but disputed by Quanjer. It is of interest to note that usually not all cells in a group become affected and even in very extreme cases there are normal, functional cells within a diseased group. The cells which thus remain normal are either parenchyma cells or sieve tubes.

Accompanying the destruction of the phloem groups and the formation of intercellular spaces are changes in the parenchyma cells of the pericycle and perimedullary zone consisting of a radial stretching of these elements and sometimes in an increase in the actual number. Occasionally the pericycle increases to such an extent as to form a band of tissue between the outer fibers and the primary phloem.

An unusual type of phloem necrosis is sometimes observed in plants affected with leafroll. The cells of the phloem are neither shrunken nor chemically changed. The walls, however, are very attenuate, in places completely obliterated, or sometimes slightly swollen (Pl. 39, B). The protoplasm is abundant and usually disorganized. Whether this condition is a step in the progress of the disease and is later followed by cutinization or other chemical changes, the writer can not state. It does not appear to be such though, since this condition is often found in fully mature plants.

The formation of intercellular spaces is not always the first disease phenomenon. In the internal region, near the fibers, a thickening of the walls of both phloem and pith cells occurs. No chemical changes have been demonstrated to accompany these swellings, and the walls remain pure cellulose. Plate 38, D, shows the beginning of cell wall thickening in a primary phloem group. Although this thickening is sometimes very extensive, the cells themselves do not seem to be harmed thereby.

Necrosis, as we have seen, is restricted to certain areas ("*differenzierete Nekrose*" of Küster; *3, p. 309*), notwithstanding the close anastomosing of the individual groups of the phloem and the direct connection of external and internal phloem through the leaf gaps. If a specific virus is the cause of potato leafroll, one would expect all phloem groups to be indiscriminately affected. Very often, however, only certain groups are diseased, and if the attack is severe the pathological condition may still be restricted either to the internal phloem or the external

phloem alone. This condition might be explained by assuming that the individual cells react differently toward the stimulus and that we have a more or less complete antagonism in those groups of cells which suffer only slightly or not at all. If, however, the toxin is localized or the virus is produced *in situ*, differential necrosis of the phloem as well as necrosis of certain cells of the cortex and pericycle becomes explainable.

The accumulation of starch in the diseased leaves, together with the reddish discoloration suggests, of course, inhibition in the process of translocation. This nonremoval of synthesized food material may be caused by lack of minerals or by the partial or complete stoppage of the path of translocation for these substances. In many instances necrosis of the phloem could in itself account for inhibition in translocation even if the phloem of petiole and midrib is mostly normal. In such cases the phloem of the stem, near the point of the insertion of the leaf, is usually severely diseased, much more so than in any other region of the stem. The nonremoval of starch from leaves where necrosis of the phloem is no factor of importance has still to be explained.

The rolling of the leaves and the characteristic xerophytic appearance of the diseased plants is the resultant of many interrelated changes and processes; such changes could not be produced by simple anatomical disturbances; nor can the results be explained on merely a mechanical basis.

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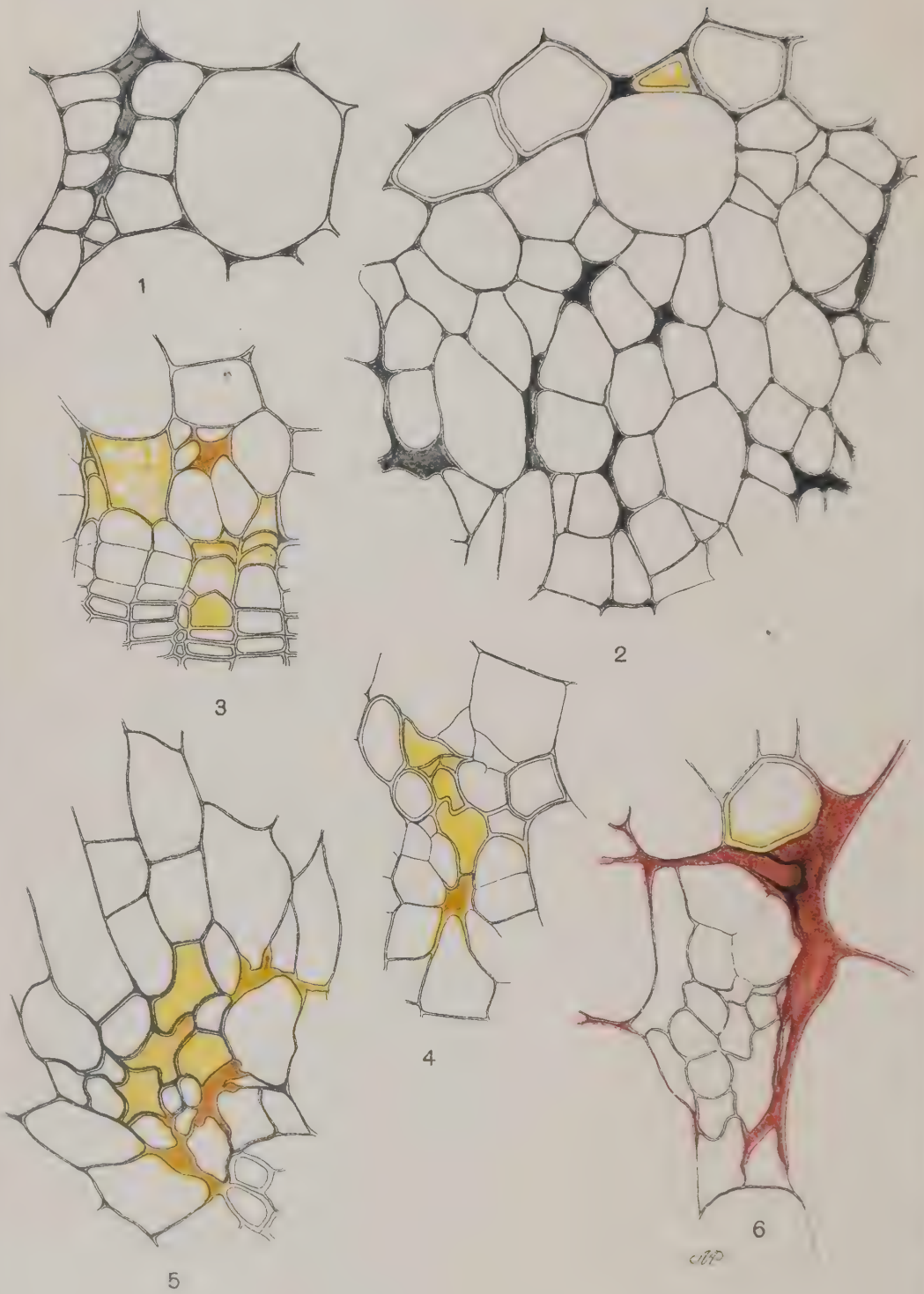


A. Hoen & Co. Lith.

PLATE C

Camera-lucida drawing of diseased tissues of the Irish potato:

1.—Tranverse section of an internal phloem group showing initial stage in formation of intercellular spaces. (Compare Plate 38, C.)

2.—Tranverse section of external phloem and pericycle showing the same condition.

3.—Transverse section of interfascicular region of mature stem showing necrosis in xylem, cambium, medullary ray cells, and cortex.

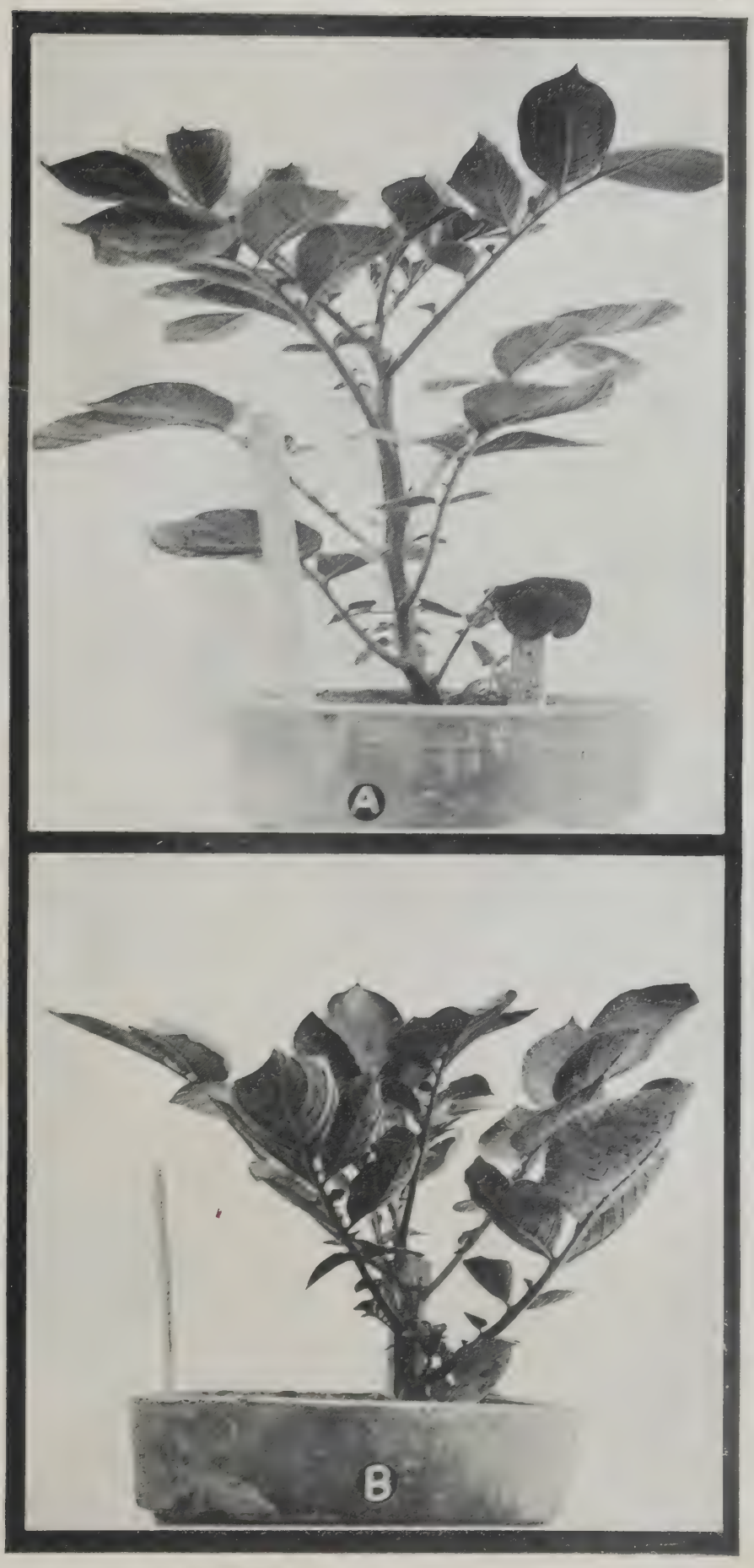
4.—Transverse section of external phloem. The cells of the phloem are filled with a granular deposit, yellow in color. Below these cells is seen a large intercellular space filled with a secretion plastic in nature.

5.—Transverse section of internal phloem of mature stem, showing severe necrosis. Note that a few cells in a group are not diseased. (Compare Pl. 44, A, B.)

6.—Transverse section of internal phloem group, showing formation of a large intercellular cavity extending up to the protoxylem and involving a few cells of the phloem. The protoxylem cell above is filled with the yellow deposit.

PLATE 35

A.—Normal potato plant. Paul Kruger variety. Ithaca, N. Y., March, 1910.
B.—Potato leafroll in Paul Kruger variety. Note especially shape of basal leaves and reduction in the length of the internodes. Ithaca, N. Y., March, 1910.


A
B

8

PLATE 36

Typical potato leafroll in Early Rose variety. Ithaca, N. Y., July 30, 1917.

PLATE 37

Typical potato leafroll in Paul Kruger variety. The plant to the left in the photograph is normal, the one to the right diseased. Ithaca, N. Y., July, 1917.

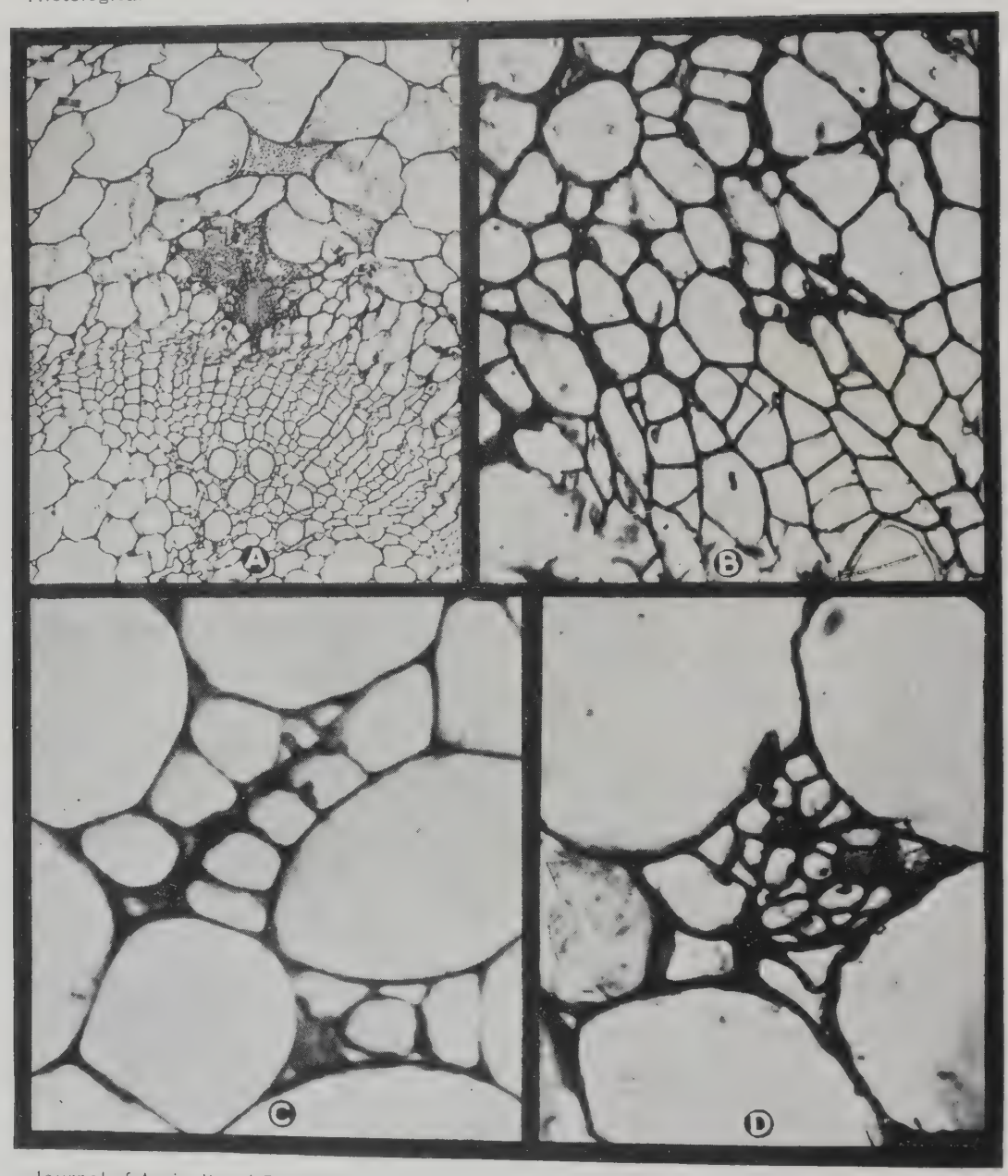

A
B
C
D

PLATE 38

A.—Cross section through upper part of stem, showing necrosis in outer phloem and cortex. x 104.

B.—Cross section through distal region of stem, showing formation of intercellular spaces in outer phloem and cortex. x 400.

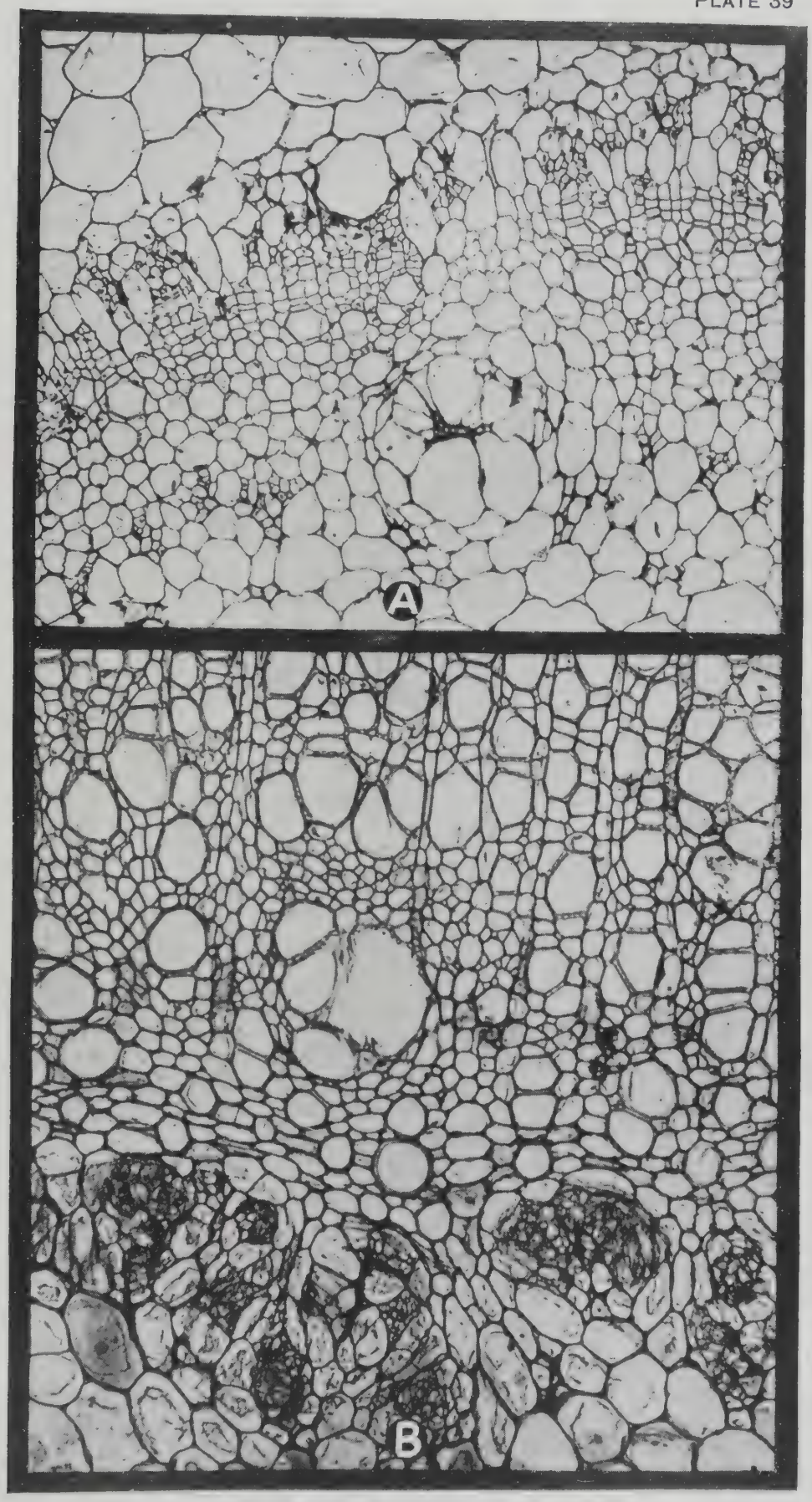
C.—Cross section through distal region of stem, showing formation of intercellular spaces between the cells of the inner phloem. x 800.

D.—View of another phloem group showing cell wall thickening. x 320.

PLATE 39

A.—Cross section through lateral bundle of petiole showing a diseased phloem group in the internal region and the effect of necrosis on the surrounding parenchyma. × 130.

B.—Cross section of stem showing an unusual type of necrosis. × 130.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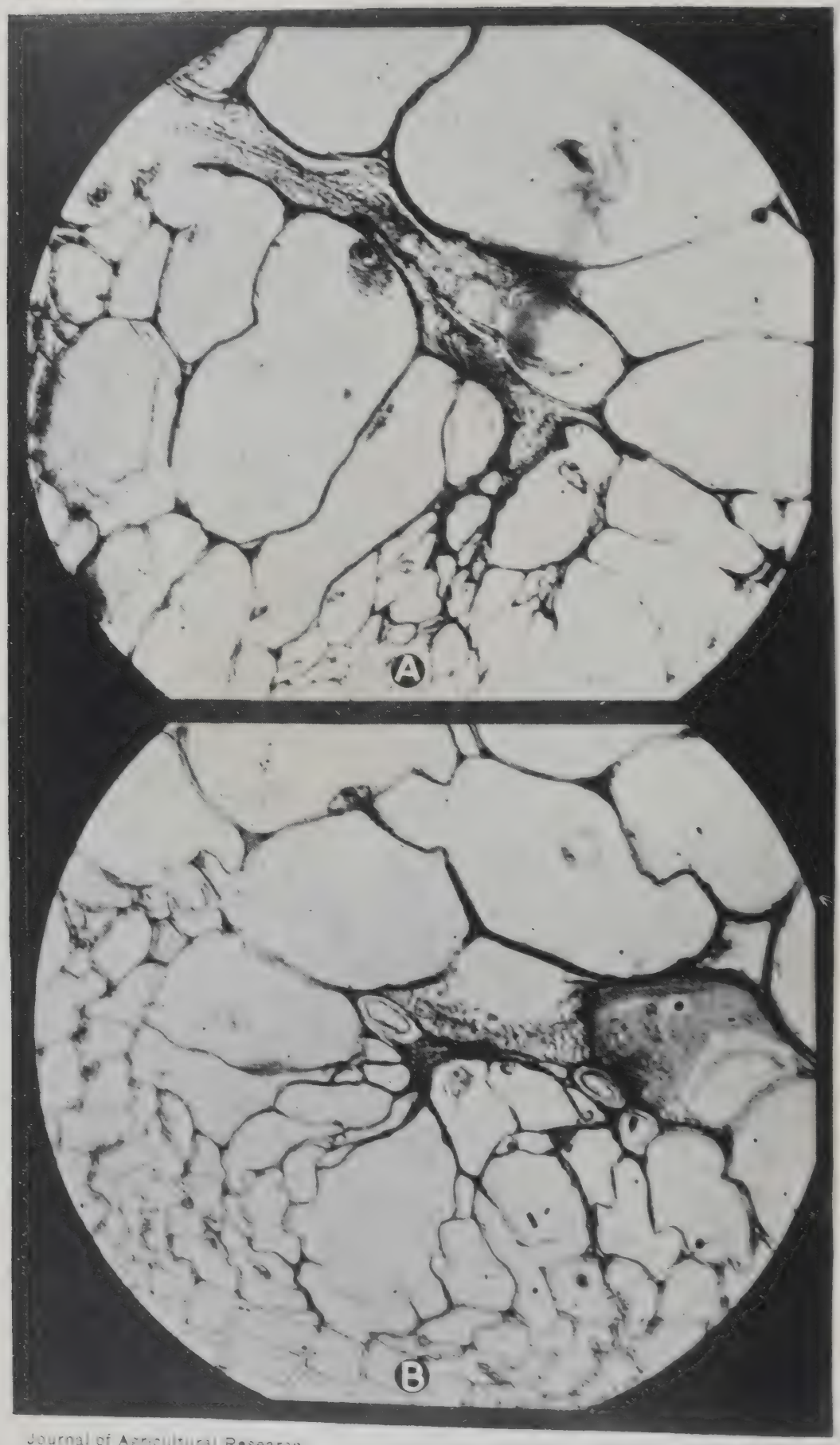
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PLATE 40

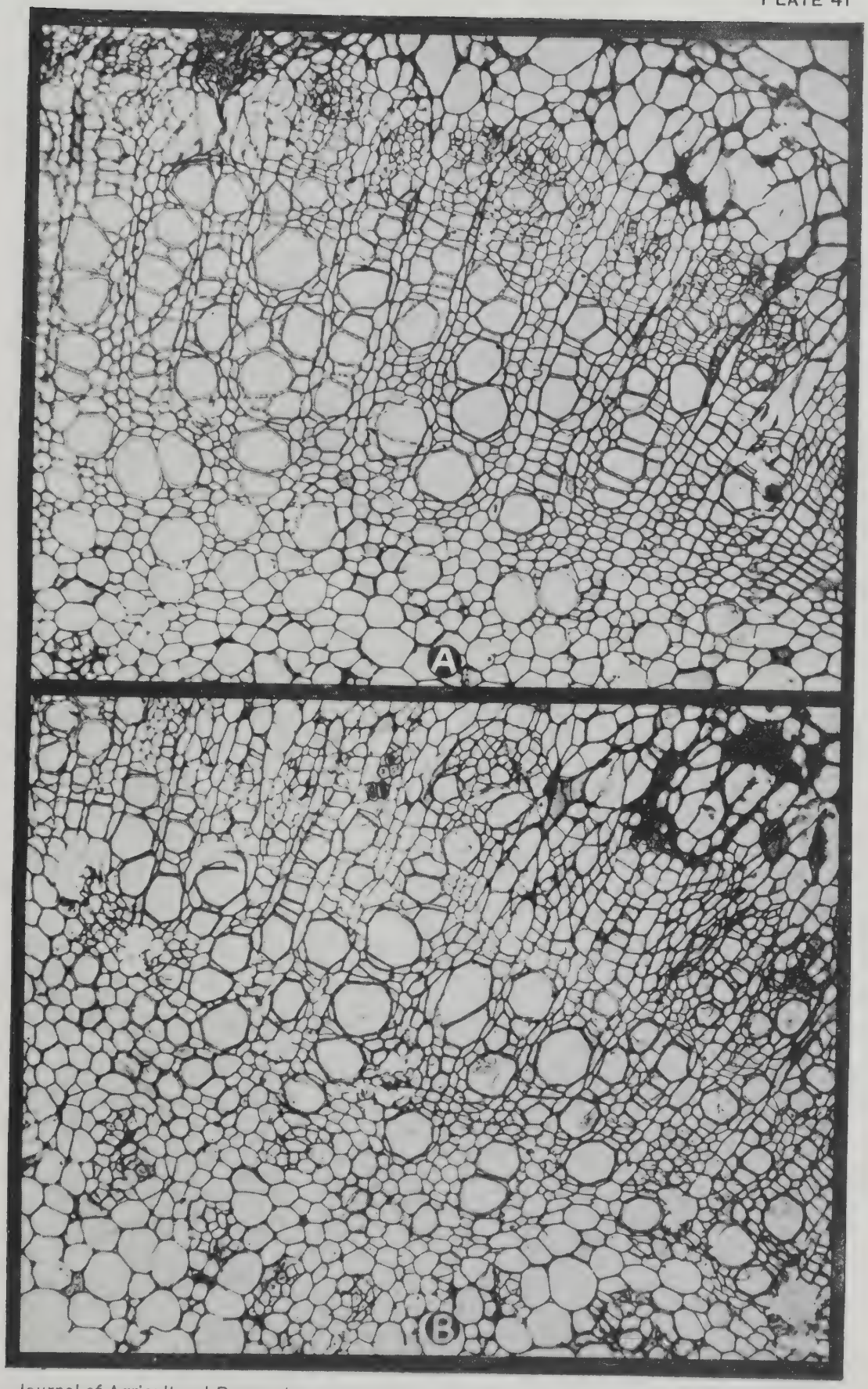
A.—Cross section of stem showing granular deposit in cell of cortex and formation of intercellular spaces proceeding from the region of the fibers centripetally. × 450.

B.—Cross section of stem showing large schizogenous cavity between cells of pericycle and cortex and centripetal advance of the formation of intercellular cavities. × 450.

PLATE 41

A.—Section of middle portion of stem showing necrosis in cells of cortex and primary phloem. × 130.

B.—Cross section of middle portion of stem showing diseased areas in pericycle and outer phloem. × 130.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A
B

PLATE 42

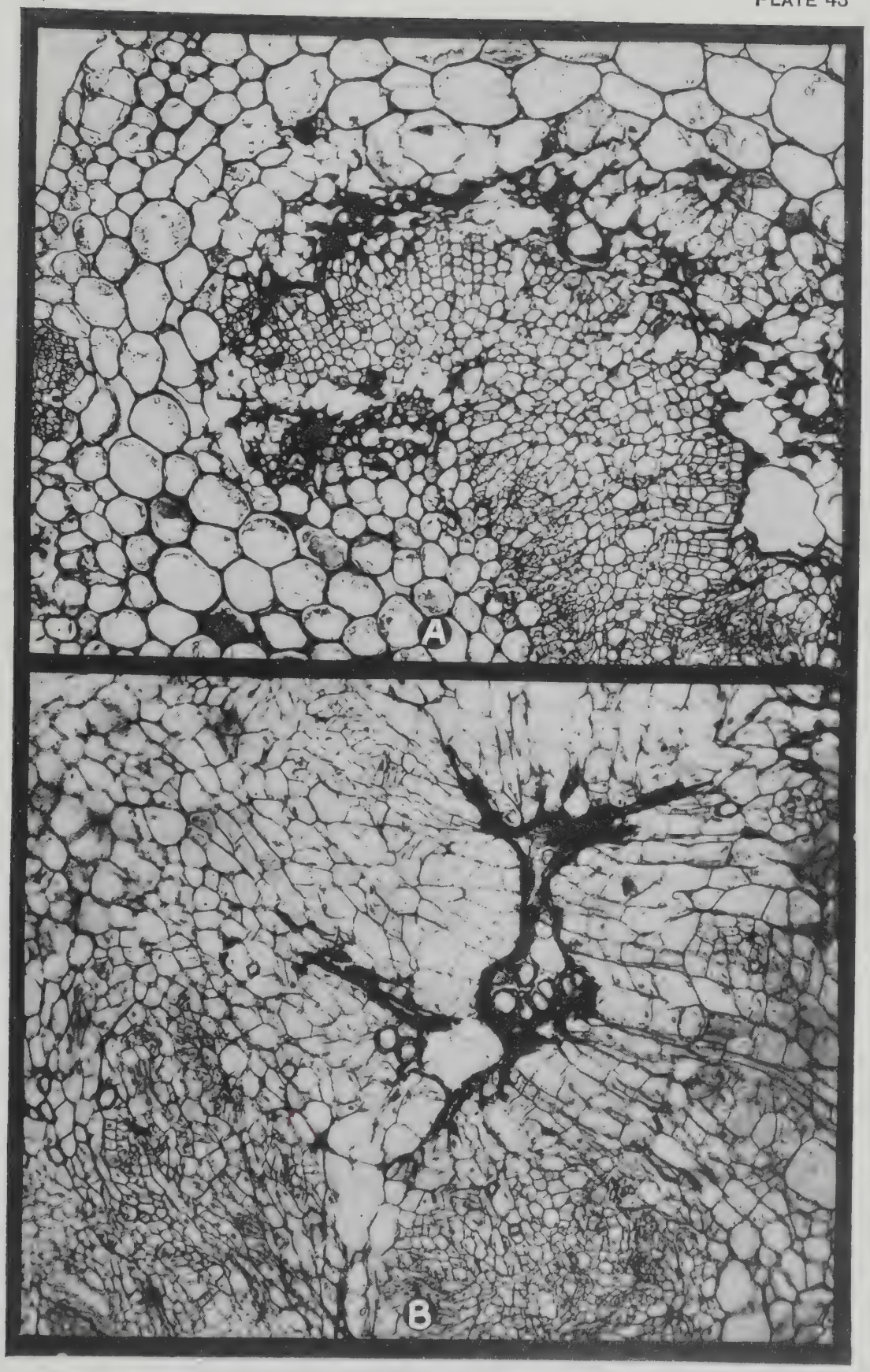
A.—Cross section of internodal region of upper part of stem showing radial stretching of the elements of the pericycle. × 130.

B.—Longitudinal section of nodal region of upper part of stem showing extent of necrosis. × 130.

PLATE 43

A.—Cross section of petiole of mature plant showing severe necrosis. All of the outer phloem and part of the inner phloem groups are destroyed. The adjacent cells of the cortex are also disorganized. × 130.

B.—Cross section of nodal region of stem tip showing formation of large lysigenous cavities extending from cortex to pith and involving inner phloem groups and metasylem. × 130.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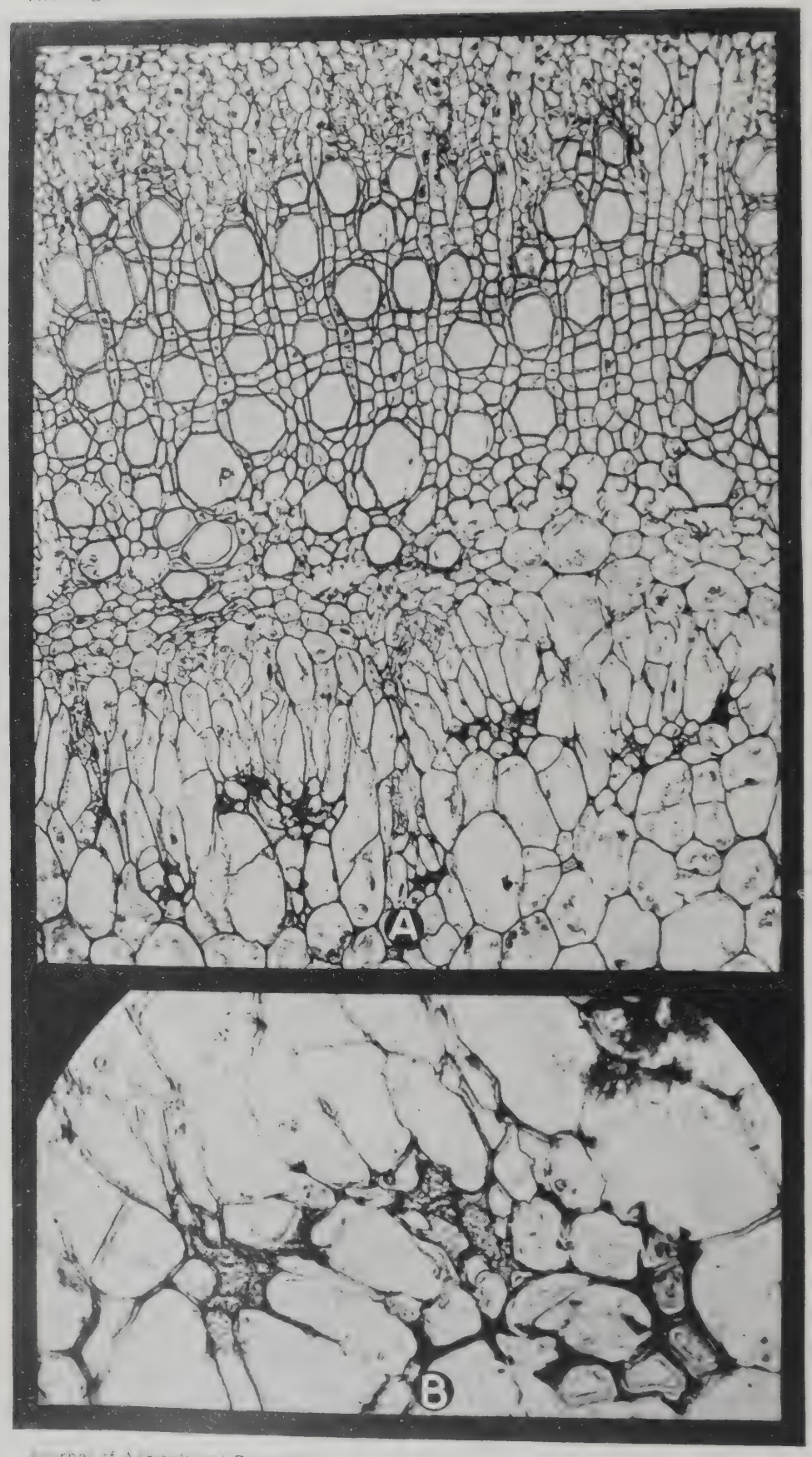
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PLATE 44

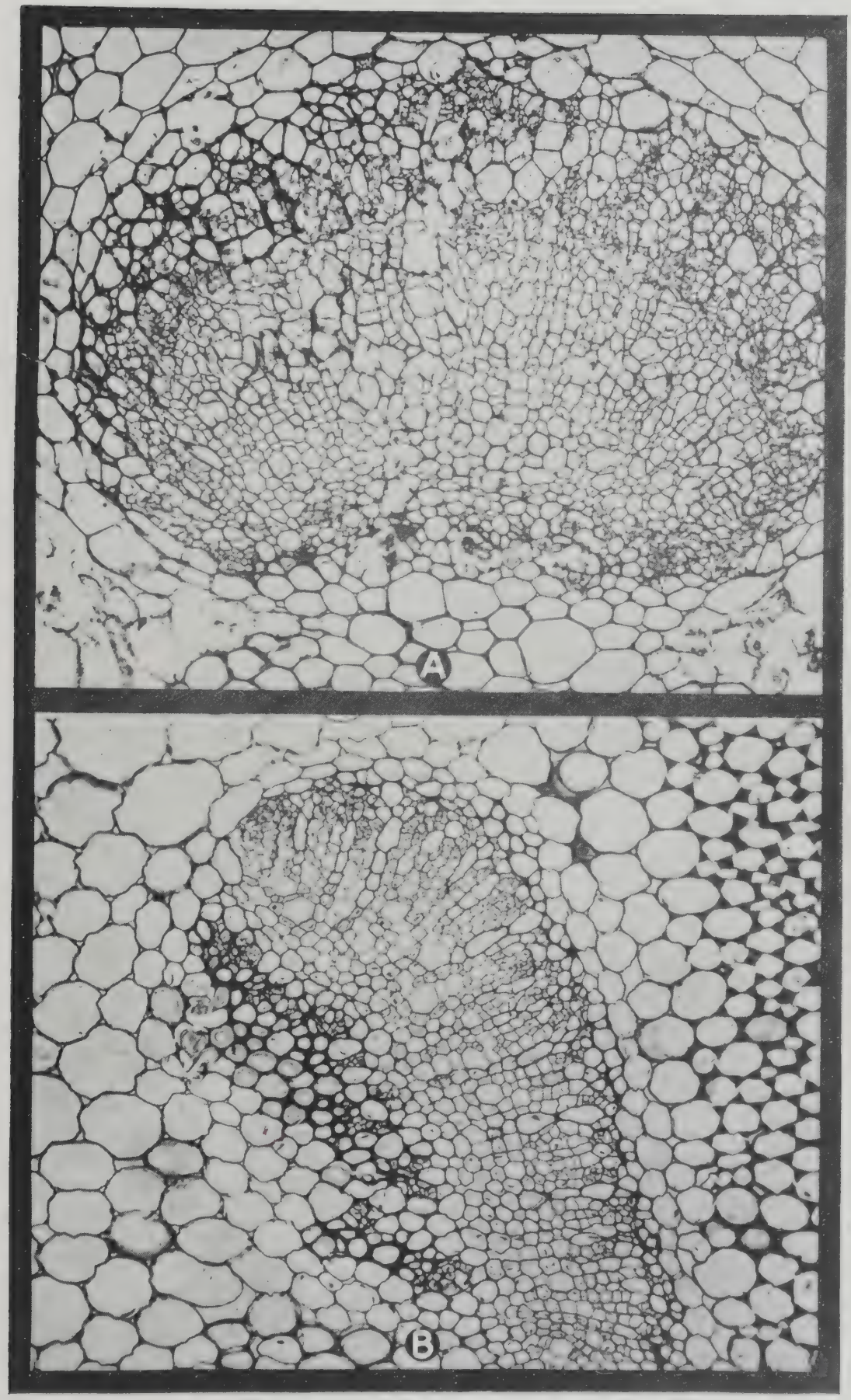
A.—Cross section of stem of mature plant, showing necrosis of internal phloem together with radial elongation of the cells of the perimedullary zone. Outer phloem is well developed and normal. × 130.

B.—Enlarged view of a necrotic internal phloem group. Almost all of the cells in a group are filled with a granular precipitate. × 400.

PLATE 45

A.—Cross section of midrib of mature plants (Magnum Bonum), showing abnormal development of the vascular tissue with thickenings of the walls of the phloem adjacent to the fibers. × 130.

B.—Cross section of petiole of mature plant, showing abnormally large development of the vascular tissue of the petiolar wings. ×130.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BACTERIOLOGICAL STUDIES ON ALFALFA SILAGE

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INTRODUCTION

The practicability of making silage from alfalfa has been under investigation at this Station for several years. During the spring of 1914 the Dairy Department erected seven small stave silos, estimated to hold 10 tons each, for experimental purposes. The silos were filled with the following combinations:

Alfalfa alone.
Alfalfa 10 parts and corn chop 1 part.
Alfalfa 20 parts and blackstrap molasses 1 part.
Alfalfa 10 parts and alfalfa-molasses feed 1 part.
Alfalfa 4 parts and straw 1 part.
Alfalfa 2 parts and green rye 1 part.
Rye alone,

In the spring of 1915 the silos were again filled, but with the following combinations:

Alfalfa alone.
Alfalfa 20 parts and blackstrap molasses 1 part.
Alfalfa 10 parts and blackstrap molasses 1 part.
Alfalfa 10 parts and corn chop 1 part.
Alfalfa 6 parts and sweet-sorghum stover 1 part.
Alfalfa 2 parts and green rye 1 part.
Rye alone.

The exact ratio of the alfalfa and supplements in some cases varied slightly from the general plan outlined. Detailed information relative to this ratio and to filling the silos may be found in the report of Reed and Fitch (*15*).[1] Chemical analysis of the silage was made each year by the Department of Chemistry, while the microbial content was determined by the Department of Bacteriology.

Reed and Fitch (*15, p. 3*) concluded from these investigations that—

(1) Alfalfa will make a fairly good quality of silage, and it will be readily eaten by cattle if fed within a few months after being siloed.

[1] Reference is made by number (italic) to "Literature cited," p. 591–592.

(2) The addition of carbohydrate material, such as corn meal, blackstrap molasses, sweet-sorghum stover, and green rye, when put into the silo resulted in preserving it for a longer time than when alfalfa was siloed alone.
(3) Of the supplements used in these experiments blackstrap molasses proved to be the best, corn chop was next in order, followed by sweet sorghum stover and green rye.
(4) The mixture of alfalfa and blackstrap molasses was the most practical one used.
(5) There is as much acid produced in alfalfa silage as in kafir or cane silage.

In a preliminary report pertaining to this investigation, Swanson and Tague (*17, p. 292*) state that—

(1) Most of the acids present in alfalfa silage are produced in the first two weeks.
(2) The addition of supplements insures a more rapid and plentiful production of acids.
(3) Sugar present in the material used in making silage disappears very rapidly. Completely matured silage contains no sugar.
(4) Molasses was the most effective supplement.
(5) Alfalfa as it is put into the silo contains only a small amount of nitrogen in amino form.
(6) Alfalfa silage contains a large amount of nitrogen in the amino form. In good silage about one-third of the nitrogen is in this form, and in bad alfalfa silage amount is sometimes one-half of the total nitrogen.
(7) Most of the change of nitrogen into amino form takes place in the first 10 days.

BACTERIOLOGICAL INVESTIGATIONS IN 1914

METHODS OF PROCEDURE

It was planned to collect samples for analyses at the time of filling the silo, each day after filling for seven days, every other day for the next week, every four days for the following two weeks, then once a week, and finally once a month until the silo was opened. While circumstances came up which interfered with this schedule at various times, this general plan of procedure was followed. The silage for analysis was obtained from the silos from a small hole about 2 inches in diameter bored into the side of the silo about 2 or 3 feet from the level of the ground. The samples of silage were collected in sterile containers by means of a large extension auger. After the required amount was obtained, the holes were tightly stoppered with paraffined corks. The general practice was to collect the samples, as aseptically as possible, from new holes each time. They were removed to the laboratory and examined immediately after collecting them.

BACTERIOLOGICAL TECHNIC

Twenty gm. of the silage were placed in 200 cc. of a sterile physiological-salt solution and thoroughly shaken. The necessary dilutions were made according to standard methods.

Plain agar was used for determining the total number of microorganisms.

Glucose-acetic-acid agar was used to determine the total number of the Bulgarian types of organisms present. The medium was 1 per cent

glucose agar, to which was added 1 cc. of a 1 per cent sterile acetic-acid solution. The acid solution was added directly to the plates and the glucose agar was mixed with it when the plates were poured. Lactose agar was used in place of glucose agar in the preliminary work, but from subsequent work it was observed that the glucose medium favored this type of organisms more than the lactose agar. The small amount of acid added was sufficient to check practically all types of organisms except the Bulgarian group and the yeasts. After a little experience there was no difficulty in distinguishing between these two types, on account of their characteristic colonies.

Litmus-lactose agar was used to determine total numbers, as well as the acid and neutral types, of microorganisms.

Bile lactose fermentation tubes were employed in determining colon organisms. The Dunham fermentation tubes were inoculated with different dilutions of the silage extract. The tube with the highest dilution showing gas production was used as an estimate of the total number of organisms of the colon group present. To substantiate further this presumptive test, different dilutions were plated out from time to time, and the organisms of the colon type isolated and identified.

Glucose fermentation tubes were used in determining the total number of yeasts present by noting gas production in the different dilution tubes. To be certain that the gas was due to yeast fermentation, stained preparations were made from each dilution and examined for the presence of yeast cells. If gas was present and no yeast could be demonstrated, it was taken for granted that the gas formation was not due to yeasts, but to other causes. The yeast count from the acid agar was used to check this method, and they compared very favorably. The general rule, however, was that higher numbers were obtained from the fermentation tubes than from the plates.

All media used were made from Liebig's beef extract, and a reaction of +1.0 to phenolphthalein required. The period of incubation, unless otherwise stated, was always four days at 37.5° C. The long period of incubation was used to favor the complete development of the Bulgarian type. The enumeration of all plates was done by the aid of a hand lens.

The principal and predominating types of microorganisms as they appeared on the different media from time to time were isolated. A morphological, cultural, and biochemical study was made from the organisms thus isolated.

Stained preparations were made directly from the silage infusion in order to check the results of the cultural analysis. It seemed unnecessary to employ the use of synthetic media after comparing the microscopic appearance of the silage infusion with the results obtained from the culture media, as the media gave a very good estimate of the true microbial content of the silage.

EXPERIMENTAL DATA

The silos were filled, beginning on May 23, 1914, with the first cutting of alfalfa, and were opened in January, 1915. The condition of the silage, when the silos were opened, is well described by Reed and Fitch (*15, p. 10–11*):

> In most cases the silage had settled five or six feet from the top of the silo and had drawn away slightly from the wall at the surface. The exceedingly dry, hot weather of 1914 perhaps caused more drying than would have occured during a normal year. The upper two feet of silage was spoiled in all the silos. The silage made from alfalfa alone was very dark in color and it had a disagreeable odor, which is characteristic of alfalfa when preserved in the silo. The mixtures of alfalfa and corn chop, alfalfa and molasses, alfalfa and molasses feed, all showed a dark-green color. All of the mixtures had a strong odor. The moisture content of all mixtures except the alfalfa and rye was very low. * * * The mixtures of alfalfa and straw, and alfalfa and molasses feed did not make a good quality of silage. On the whole, the results obtained in this trial were not satisfactory.

The palatability test as determined by the Dairy Department demonstrated that cattle prefer the mixtures in the following order: Alfalfa and molasses, alfalfa and corn chop, alfalfa alone, alfalfa and rye, alfalfa and molasses feed, alfalfa and straw, and rye alone.

The acidity of the silage as determined by the Chemistry Department indicated that all the material underwent a normal acid fermentation. The acidity for the silage, when the silos were opened was as follows: Alfalfa alone 1.63 per cent, alfalfa and corn chop 1.84 per cent, alfalfa and molasses 2.28 per cent, alfalfa and molasses feed 2.03 per cent, alfalfa and straw 1.37 per cent, alfalfa and rye 1.24 per cent, and rye alone 2.79 per cent.

The results obtained from the bacteriological analyses are given in Table I.

In general, the bacteriological data were unsatisfactory. Little evidence was gathered to account for the differences noted in the quality of the various kinds of silage. The microbial flora of alfalfa silage is very similar to that of silage made from the common forage crops, the four principal types of organisms being (1) acid producers, (2) yeasts, (3) organisms of the colon group, and (4) miscellaneous. As in normal silage, the acid producers, as a rule, predominated in alfalfa silage. Yeasts and the organisms of the colon group had a tendency to coincide in their course of development in the silage. It has been observed from the study of the fermentation processes in a good quality of silage that these two groups rapidly increase in numbers for the first few days, then gradually diminish. While the same tendency was indicated in the different kinds of alfalfa silage, it was more marked in some than in others.

TABLE I.—*Microbial content of various kinds of silage—Experiments of 1914*

ALFALFA ALONE

Date.	Days.	Number of microorganisms per gram upon—					Number of organisms of colon type per gram.	Number of yeasts per gram.
		Plain agar—Total number.	Litmus-lactose agar.			Acid agar—Acid type.		
			Total.	Acid producers.	Neutral type.			
May 23	0	3,000,000	520,000	380,000	140,000	0	10,000	10,000
May 25	2	43,000,000	48,000,000	46,000,000	2,000,000	285,000	1,000,000	10,000,000
May 26	3	25,000,000	.....	.....	.....	7,000	1,000	1,000,000
May 27	4	.....	10,000,000	9,000,000	1,000,000	0	10,000	1,000,000
May 28	5	.....	.....	.....	.....	70,000	10,000	10,000
May 29	6	17,000,000	14,000,000	14,000,000	0	42,000	1,000	10,000
May 30	7	8,500,000	5,000,000	5,000,000	0	300,000	100	10,000
June 1	9	16,000,000	5,500,000	4,500,000	1,000,000	4,000	10	1,000
June 2	10	10,000,000	8,000,000	8,000,000	0	120,000	100	1,000
June 3	11	11,000,000	.....	.....	.....	400,000	100	1,000
June 4	12	7,000,000	5,500,000	5,500,000	500,000	24,000	0	100
June 5	13	8,000,000	10,000,000	8,000,000	2,000,000	500,000	1,000	1,000
June 6	14	4,000,000	3,000,000	2,500,000	500,000	10,000	10	10
June 8	16	17,000,000	10,000,000	5,000,000	5,000,000	4,000	10	10
June 9	17	500,000	600,000	300,000	300,000	.....	10	10
June 10	18	10,000,000	13,000,000	12,000,000	1,000,000	.....	100	10
June 11	19	2,000,000	2,000,000	1,800,000	200,000	20,000	0	100
June 15	23	10,000,000	800,000	400,000	400,000	18,000	0	10
June 17	25	.....	.....	.....	.....	22,000	100	100
June 19	27	11,000,000	10,000,000	600,000	9,400,000	12,000	10	100
June 22	30	30,000,000	9,500,000	8,000,000	1,500,000	500,000	10,000	10,000
June 26	34	30,000,000	500,000,000	200,000,000	300,000,000	150,000	0	0
July 3	41	4,500,000	3,000,000	2,500,000	500,000	50,000	1,000	1,000
July 10	48	1,500,000	1,600,000	1,200,000	400,000	2,000	0	0
July 17	55	300,000	300,000	0	300,000	4,000	10	10
July 28	66	10,000,000	30,000,000	22,000,000	8,000,000	2,000	.....	10
September 18	118	.....	.....	.....	.....	450,000	10	10
October 2	132	500,000,000	500,000,000	250,000,000	250,000,000	550,000	10,000	10,000
October 30	160	50,000,000	30,000,000	5,000,000	25,000,000	2,000,000	10,000	10,000

TABLE I.—*Microbial content of various kinds of silage—Experiments of 1914*—Continued

ALFALFA AND CORN CHOP

Date.	Days.	Number of microorganisms per gram upon—					Number of organisms of colon type per gram.	Number of yeasts per gram.
		Plain agar—Total number.	Litmus-lactose agar.			Acid agar—Acid type.		
			Total.	Acid producres.	Neutral type.			
May 25	0	63,000,000	78,000,000	0	73,000,000	700	10,000	10,000
May 26	1	87,000,000	38,000,000	0	33,000,000	0	10,000	10,000
May 27	2	.....	7,000,000	2,000,000	5,000,000	5,000	1,000	1,000
May 28	3	15,000,000	.....	.....	.....	120,000	10,000	10,000
May 29	4	10,000,000	17,000,000	15,000,000	2,000,000	67,000	1,000,000	1,000,000
May 30	5	29,000,000	27,000,000	5,000,000	22,000,000	300,000	100,	1,000
June 1	7	.....	9,000,000	8,500,000	500,000	50,000	100	100
June 2	8	30,000,000	20,000,000	20,000,000	0	14,000	100	10,000
June 3	9	5,000,000	.....	.....	.....	100,000	100,	1,000
June 4	10	6,500,000	20,000,000	10,000,000	10,000,000	18,000	100	100
June 5	11	8,000,000	6,000,000	2,000,000	4,000,000	60,000	10	100
June 6	12	80,000,000	50,000,000	35,000,000	15,000,000	1,000	10	10
June 8	14	13,000,000	18,000,000	7,500,000	10,500,000	50,000	100	100
June 9	15	600,000	5,000,000	3,000,000	2,000,000	3,000	100	10
June 10	16	2,000,000	2,500,000	2,000,000	500,000	120,000	0	0
June 11	17	10,000,000	11,000,000	9,000,000	2,000,000	15,000	1,000	10
June 15	21	400,000	500,000	100,000	400,000	200,000	0	100
June 17	23	.....	8,500,000	7,000,000	1,500,000	1,600,000	10,000	10,000
June 19	25	7,000,000	6,000,000	4,500,000	1,500,000	100,000	100	100
June 22	28	10,000,000	7,000,000	5,500,000	1,500,000	10,000	10	10
June 26	32	15,000,000	9,000,000	3,000,000	6,000,000	20,000	0	0
July 3	39	10,000,000	9,500,000	7,500,000	3,000,000	200	0	0
July 10	46	400,000	500,000	200,000	300,000	200	0	0
July 17	53	1,000,000	1,300,000	300,000	1,000,000	0	100	100
July 23	64	600,000	190,000	0	190,000	100	0	10
September 18	116	3,000,000	900,000	100,000	800,000	200,000	1,000	1,000
October 2	130	170,000,000	230,000,000	30,000,000	200,000,000	60,000	10	100
October 30	158	200,000,000	200,000,000	50,000,000	150,000,000	120,000	100	1,000

ALFALFA AND MOLASSES

May 26	0	.....	.....	.....	.....	0	1,000	1,000
May 27	1	.....	90,000,000	85,000,000	5,000,000	0	10,000	1,000,000
May 28	2	8,000,000	.....	.....	.....	300,000	10,000	10,000
May 29	3	31,000,000	18,000,000	17,000,000	1,000,000	12,000	1,000	1,000
May 30	4	30,000,000	33,000,000	32,000,000	1,000,000	2,500,000	1,000	10,000
June 1	6	.....	2,000,000	1,500,000	500,000	250,000	10	100
June 2	7	23,000,000	28,000,000	28,000,000	0	10,000	1,000	10,000

June 3	8	27,000,000	.....	.....	.....	400,000	10,000	10,000
June 4	9	70,000,000	40,000,000	20,000,000	20,000,000	200,000	0	100
June 5	10	8,000,000	7,000,000	5,000,000	2,000,000	75,000	10	100
June 6	11	14,000,000	4,000,000	4,000,000	0	50,000	10	10
June 8	13	12,000,000	3,500,000	3,500,000	0	70,000	10	10
June 9	14	4,000,000	600,000	500,000	100,000	110,000	10	100
June 10	15	12,000,000	13,000,000	10,000,000	3,000,000	150,000	1,000	100
June 11	16	.....	1,000,000	1,000,000	0	75,000	0	0
June 15	20	5,000,000	7,500,000	1,000,000	6,500,000	300,000	10	100
June 17	22	.....	.....	.....	.....	800,000	1,000,000	1,000,000
June 19	24	2,000,000	5,500,000	5,500,000	0	300,000	0	100
June 22	27	900,000	200,000	200,000	0	600,000	0	0
June 26	31	400,000	300,000	0	300,000	31,000	0	0
July 3	38	50,000	20,000	10,000	10,000	1,100	0	0
July 10	45	45,000	50,000	40,000	10,000	200	0	0
July 17	52	1,300,000	400,000	0	400,000	0	0	0
July 18	63	100,000	.....	.....	.....	0	0	0
September 18	115	2,000,000,000	375,000,000	0	375,000,000	150,000	100	10
September 2	129	250,000	20,000	0	20,000	40,000	0	0
October 30	157	200,000,000	175,000,000	10,000 000	165,000,000	250,000	10,000	10,000

ALFALFA AND ALFALFA-MOLASSES FEED

May 27	0	.....	4,000,000	1,000,000	3,000,000	0	100	1,000
May 28	1	67,000,000	.....	.....	.....	11,000	1,000,000	1,000,000
May 29	2	160,000,000	1,000,000,000	700,000,000	300,000,000	60,000	10,000	10,000
May 30	3	26,000,000	48,000,000	30,000,000	18,000,000	2,000,000	100	10
June 1	5	.....	40,000,000	15,000,000	25,000,000	3,000,000	1,000	1,000
June 2	6	16,000,000	65,000,000	65,000,000	0	100,000	1,000	1,000
June 3	7	5,000,000	.....	.....	.....	600,000	10	100
June 4	8	15,000,000	23,000,000	14,000,000	9,000,000	300,000	0	100
June 5	9	15,000,000	18,000,000	14,000,000	4,000,000	100,000	100	100
June 6	10	35,000,000	20,000,000	15,000,000	5,000,000	5,000	10	10
June 8	12	15,000,000	6,000,000	4,500,000	1,500,000	200,000	10	10
June 9	13	1,600,000	4,000,000	2,000,000	2,000,000	100,000	100	100
June 10	14	.....	8,000,000	6,000,000	2,000,000	75,000	100	1,000
June 11	15	4,000,000	1,700,000	1,000,000	700,000	.....	0	100
June 15	19	1,500,000	1,000,000	200,000	800,000	600,000	10	10
June 17	21	.....	.....	.....	.....	300,000	10,000	1,000,000
June 19	23	10,000,000	30,000,000	20,000,000	10,000,000	7,500,000	1,000	10,000
June 22	26	3,000,000	4,000,000	3,500,000	500,000	200,000	0	0
June 26	30	3,500,000	5,000,000	2,000,000	3,000,000	150,000	10	1,000
July 3	37	1,500,000	500,000	0	500,000	5,000	0	0
July 10	44	1,000,000	.....	.....	.....	0	100	0
July 17	51	1,600,000	1,000,000	0	1,000,000	0	1,000	1,000
July 28	62	500,000	200,000	0	200,000	0	100	100
September 18	114	2,000,000	3,000,000	1,250,000	1,750,000	60,000	10	100
October 2	128	7,000,000	5,000,000	0	5,000,000	40,000	0	1,000
October 30	156	25,000,000	30,000,000	5,000,000	25,000,000	20,000	10	10

TABLE I.—*Microbial content of various kinds of silage—Experiments of 1914*—Continued

ALFALFA AND STRAW

Date.	Days.	Number of microorganisms per gram upon—					Number of organisms of colon type per gram.	Number of yeasts per gram.
		Plain agar—Total number.	Litmus-lactose agar.			Acid agar—Acid type.		
			Total.	Acid producers.	Neutral type.			
May 27	0	122,000,000	.....	.....	.....	0	10,000	.....
May 28	1	.....	.....	.....	.....	30,000	1,000,000,000	1,000,000,000
May 29	2	75,000,000,000	70,000,000,000	60,000,000,000	10,000,000,000	1,000,000	1,000,000,000	10,000,000,000
May 30	3	20,000,000,000	50,000,000,000	40,000,000,000	10,000,000,000	6,000,000	10,000,000	1,000,000,00
June 1	5	1,000,000,000	1,500,000,000	1,000,000,000	500,000,000	700,000	1,000,000	10,000,000
June 2	6	800,000,000	15,000,000,000	14,000,000,000	1,000,000,000	100,000	10,000	1,000,000
June 3	7	4,000,000,000	.....	.....	.....	800,000	10,000	1,000,000
June 4	8	470,000,000	600,000,000	600,000,000	0	2,200,000	10,000	1,000,000
June 5	9	300,000,000	580,000,000	580,000,000	0	5,200,000	10,000	10,000
June 6	10	2,000,000,000	2,000,000,000	1,250,000,000	150,000,000	500,000	1,000	10,000
June 8	12	230,000,000	300,000,000	250,000,000	50,000,000	175,000	1,000	10,000
June 9	13	450,000,000	500,000,000	480,000,000	20,000,000	40,000	10,000	10,000
June 10	14	.....	70,000,000	70,000,000	0	240,000	1,000	1,000
June 11	15	370,000,000	70,000,000	60,000,000	10,000,000	15,000	10,000	10,000
June 15	19	80,000,000	90,000,000	90,000,000	0	400,000	100	10,000
June 17	21	.....	1,500,000	1,200,000	300,000	200,000	10,000	1,000,000
June 19	23	200,000,000	50,000,000	150,000,000	200,000,000	3,500,000	1,000,000	1,000,000
June 22	26	10,000,000	40,000,000	40,000,000	0	1,000,000	100	100
June 26	30	16,000,000	18,000,000	10,000,000	8,000,000	100,000	100	100
July 3	37	30,000,000	40,000,000	20,000,000	20,000,000	5,000	1,000	1,000
July 10	44	2,500,000	1,000,000	500,000	500,000	0	100	100
July 17	51	1,500,000	500,000	0	0	500	1,000	1,000
July 28	62	2,000,000	2,000,000	1,000,000	1,000,000	0	0	100
September 18	114	3,000,000	2,000,000	400,000	1,600,000	8,000,000	1,000	1,000
October 2	128	7,000,000	900,000	0	900,000	8,000	10	10,000
October 30	156	25,000,000	20,000,000	5,000,000	15,000,000	10,000	10	1,000

ALFALFA AND RYE

May 28	0	15,000,000,000	10,000,000,000	34,000,000,000	24,000,000,000	13,000	1,000,000,000	10,000,000,000
May 29	1	500,000,000	400,000,000	300,000,000	100,000,000	19,000,000	10,000,000	10,000,000
May 30	2	1,200,000,000	2,700,000,0000	1,700,000,000	1,000,000,000,	20,000,000	10,000,000	10,000,000
June 1	4	20,000,000	45,000,000	25,000,000	20,000,000	1,000,000	10,000	1,000
June 2	5	85,000,000	50,000,000	45,000,000	5,000,000	3,000,000	10,000,000	10,000,000

June 3	6	75,000,000	.....	.....	.....	8,500,000	1,000,000	10,000,000
June 4	7	250,000,000	150,000,000	60,000,000	90,000,000	7,500,000	1,000	10,000
June 5	8	50,000,000	20,000,000	2,000,000	18,000,000	1,200,000	100	100
June 6	9	750,000,000	8,000,000,000	400,000,000	7,600,000,000	12,000	10,000	10,000
June 8	11	230,000,000	80,000,000	40,000,000	40,000,000	1,200,000	10,000	10,000
June 9	12	22,000,000	30,000,000	0	30,000,000	1,800,000	10,000	10,000
June 10	13	.....	20,000,000	0	20,000,000	6,000,000	100	1,000
June 11	14	1,000,000,000	1,000,000,000	500,000,000	500,000,000	6,000,000	10,000	10,000
June 15	18	36,000,000	30,000,000	0	30,000,000	11,000,000	0	0
June 17	20	.....	6,000,000	0	6,000,000	14,000,000	0	100
June 19	22	3,000,000	350,000,000	50,000,000	300,000,000	7,500,000	10	100
June 22	25	20,000,000	20,000,000	0	20,000,000	200,000	10	100
June 26	29	10,000,000	10,000,000	0	10,000,000	600,000	0	0
July 3	36	3,500,000	2,600,000	200,000	2,400,000	16,000	100	100
July 10	43	3,000,000	2,500,000	0	2,500,000	0	0	0
July 17	50	5,000,000	5,000,000	2,000,000	3,000,000	3,000	10	1,000
July 28	61	3,000,000	6,000,000	0	6,000,000	0	0	10
September 18	113	17,000,000	8,000,000	0	8,000,000	50,000	10	100
October 2	127	37,000,000	12,000,000	0	12,000,000	30,000	0	100
October 30	155	10,000,000	5,000,000	3,000,000	2,000,000	50,000	0	0

RYE

May 28	0	60,000,000	33,000,000	18,000,000	15,000,000	3,500	10,000	10,000,000
May 29	1	250,000,000	200,000,000	190,000,000	10,000,000	9,500,000	1,000,000	10,000,000
May 30	2	700,000,000	3,000,000,000	2,000,000,000	1,000,000,000	100,000,000	1,000,000	1,000,000,000
June 1	4	300,000,000	200,000,000	100,000,000	100,000,000	12,000,000	1,000,000	10,000,000
June 2	5	50,000,000	58,000,000	58,000,000	0	4,000,000	10,000	10,000
June 3	6	85,000,000	.....	.....	.....	40,000,000	10,000	10,000
June 4	7	60,000,000	29,000,000	16,000,000	13,000,000	6,500,000	10,000	10,000
June 5	8	35,000,000	6,500,000	4,500,000	2,000,000	3,000,000	10,000	10,000
June 6	9	55,000,000	25,000,000	12,000,000	13,000,000	1,500,000	10,000	10,000
June 8	11	20,000,000	2,500,000	1,500,000	1,000,000	1,500,000	10,000	1,000,000
June 9	12	15,000,000	1,000,000	900,000	100,000	400,000	1,000	1,000
June 10	13	.....	24,000,000	23,500,000	500,000	600,000	10,000	10,000
June 11	14	35,000,000	10,000,000	10,000,000	0	2,000,000	10	1,000
June 15	18	5,000,000	1,800,000	1,000,000	800,000	2,000,000	1,000,000	10,000
June 17	20	.....	20,000,000	10,000,000	10,000,000	8,500,000	1,000,000	1,000,000
June 19	22	900,000,000	450,000,000	430,000,000	20,000,000	40,000,000	1,000,000	1,000,000
June 22	25	.....	60,000,000	40,000,000	20,000,000	5,000,000	1,000,000	10,000
June 26	29	3,500,000	7,000,000	6,000,000	1,000,000	6,000,000	10,000	10,000
July 3	36	8,000,000	9,000,000	8,500,000	500,000	40,000	1,000,000	1,000,000
July 10	43	1,500,000	400,000	300,000	100,000	25,000	10	100
July 17	50	6,000,000	1,300,000	400,000	900,000	15,000	10,000	10,000
July 28	61	1,000,000	35,000	27,000	8,000	10,000	10	10,000
September 18	113	2,000,000,000	80,000,000	30,000,000	50,000,000	40,000	100	.....
October 2	127	5,000,000	2,500,000	1,000,000	1,500,000	4,000,000	.....	.....
October 30	155	45,000,000	20,000,000	15,000,000	5,000,000	10,000,000	1,000	1,000

It has likewise been demonstrated from the study of many classes of silage of high quality that the general course of development of the total microbial flora is slow to rapid, usually covering a period of a few days to two weeks, followed by a gradual decline. The data in Table I indicate a like tendency, but the characteristic development is not so pronounced as would be expected from a good grade of silage. This is partially explained, however, by the method used in sampling and also by the small capacity of the silos used, which did not favor optimum silage fermentation. The method of sampling, no doubt, accounts for a large portion of the variations noted, since the method did not always allow the collecting of proportionate amounts of leaves and stem, or where supplements were added of proportionate amounts of alfalfa and the supplement.

BACTERIOLOGICAL INVESTIGATIONS IN 1915

The seven silos were again filled in the spring of 1915 with alfalfa and the supplements previously mentioned. The general plan of study was the same as for 1914.

However, glucose-litmus broth was used to determine the total number of acid producers. It was prepared by using 1 per cent glucose broth to which a few drops of litmus solution had been added. Several tubes of this medium were inoculated with different dilutions of the silage infusion and incubated. The total acid producers were determined by noting the acid reaction in the highest dilution present.

This method was used in place of a litmus-agar medium because a more accurate estimate of the acid producers could be obtained. If on a litmus-agar plate the acid colonies are few and other types predominate, the colonies may fail to appear acid on account of the neutralization of the acid by the alkaline by-products from the other types. The organisms in the glucose-broth solution are not held in one place, as they are in the solid-agar media. This, together with the fact that the glucose broth acts as an enrichment medium for the acid group, gives better opportunity for the acid bacteria to increase more rapidly than the miscellaneous organisms. Likewise, the acid produced from the more rapid-growing acid bacteria is sufficient to check the slower development of the miscellaneous types. The dilution method is more tedious, but, provided the differences between the dilutions are small, the results obtained are more satisfactory and more accurate. Plain gelatin was used to determine the protein-digesting types. Not having a satisfactory place for the incubation of gelatin plates, sterile tubes of gelatin were inoculated with varying dilutions of silage, and incubated at 37.5° C. for five days. The number of liquefiers were determined by placing the gelatin tubes in the ice box, after their removal from the incubator. From previous experience it has been found that a tube of digested gelatin will not solidify on cooling. Hence, by noting the highest dilution showing liquefaction after cooling, the number of liquefiers can be esti-

mated. By the use of small dilutions and more tubes, the results are probably more accurate than those obtained from the usual method of counting the liquefying colonies on gelatin plates.

EXPERIMENTAL DATA

The silos were opened on December 20, 1915, six and one-half months after being filled. The silage from all silos was of much better quality than that obtained the previous year. This was due, no doubt, to the greater care taken in packing and filling the silos (*15, p. 13–15*) and also to the supplements used. The strong offensive odor common to the silage produced in the first year's work was present only in the alfalfa and in the alfalfa-and-rye silage. The palatability test as determined by the Dairy Department demonstrated the feeding quality of the silage to be as follows: Alfalfa and molasses 20 to 1, alfalfa to molasses 10 to 1, alfalfa and corn chop, alfalfa and sweet-sorghum butts, alfalfa and rye, alfalfa alone, and rye alone.

The data obtained by the Chemistry Department, published by Swanson and Tague (*17*), failed to show the relative differences between the different kinds of silage. The alfalfa silage, like that in the previous year's work, exhibited the lowest acid content. The greatest, and practically the only characteristic difference observed from the chemical data was the total amount of acid produced. The acidity of alfalfa silage, calculated as lactic acid, on the last day examined, when 211 days old, was 1.72 per cent; alfalfa and molasses 20 to 1, 208 days old, 2.89 per cent; alfalfa and molasses 10 to 1 206 days old, 3.55 per cent; alfalfa and corn chop 10 to 1, 204 days old, 3.36 per cent; alfalfa and sweet-sorghum straw 6 to 1, 198 days old, 2.19 per cent; alfalfa and rye 2 to 1, 198 days old, 2.5 per cent; rye alone, 198 days old, 1.95 per cent,

The calculated percentage of amino nitrogen failed to exhibit any characteristic difference between the various types of silage.

The bacteriological results are tabulated in Table II.

TABLE II.—*Microbial content of various kinds of silage—Experiments of 1915*

ALFALFA

Date.	Days.	Number of microorganisms per gram of silage.					
		Total.	Total acid producers.	Bulgarian group.	Gelatin liquefiers.	Colon type.	Yeasts.
May 17.....	0	575,000	1,000,000	0	1,000	1,000	100
18.....	1	40,000.000	100,000	2,000	1,000	1,000,000	300
19.....	2	300,000,000	100,000	10,000	1,000	1,000,000	1,000
21.....	4	160,000,000	100,000,000	3,000,000	1,000	1,000,000	1,000
24.....	7	640,000,000	1,000,000,000	64,000,000	10,000	1,000,000	1,000,000
26.....	9	250,000,000	100,000,000	80,000,000	10,000	1,000	10,000
29.....	12	100,000,000	1,000,000,000	90,000,000	1,000	1,000	1,000
June 1.....	15	120,000,000	1,000,000,000	100,000,000	10,000	100	0
5.....	19	75,000,000	100,000,000	45,000,000	1,000	0	0
12.....	26	60,000,000	100,000,000	50,000,000	10,000	0	100
18.....	32	24,000,000	10,000,000	10,000,000	1,000,000	0	10,000
July 2.....	46	75,000,000	10,000,000	2,000,000	10,000	0	1,000
Aug. 6.....	81	24,000,000	100,000,000	2,000,000	10,000,000	0	10,000
28.....	103	60,000,000	100,000,000	8,000	10,000	0	100
Oct. 16	152	30,000	1,000	.....	1,000	0	100
Dec. 20.....	217	1,000,000	100,000	800	100	0	1,000

TABLE 11.—*Microbial content of various kinds of silage—Experiments of 1915*—Contd.

ALFALFA AND MOLASSES 20 TO 1

Date.	Days.	Number of microorganisms per gram of silage.					
		Total.	Total acid producers.	Bulgarian group.	Gelatin liquefiers.	Colon type.	Yeasts.
May 19.....	0	4,000,000	.....	0	1,000	10,000	100,000
20.....	1	70,000,000	1,000,000,000	290,000	.....	1,000,000	1,000,000
21.....	2	200,000,000	10,000,000	3,000,000	100,000	10,000,000	10,000
24.....	5	900,000,000	1,000,000,000	25,000,000	1,000	1,000,000	10,000,000
26.....	7	400,000,000	1,000,000,000	300,000,000	.....	0	0
29.....	10	100,000,000	100,000,000	75,000,000	100	0	100
June 1.....	13	18,000,000	1,000,000,000	20,000,000	100,000	1,000	1,000
5.....	17	40,000,000	10,000,000	40,000,000	10,000	1,000	100
12.....	24	50,000,000	100,000,000	30,000,000	1,000	100	1,000
18.....	30	3,000,000	10,000,000	3,000,000	1,000	0	0
July 2.....	44	10,000,000	1,000,000	1,000,000	1,000	0	0
Aug. 6.....	97	400,000	10,000,000	3,000,000	10,000	0	0
28.....	101	520,000	1,000,000	1,000	100,000	0	0
Oct. 16.....	150	15,000	1,000	11,000	10,000	0	1,000
Dec. 20.....	215	50,000,000	100,000	5,000,000	10,000	0	0

ALFALFA AND MOLASSES 10 TO 1

May 22.....	0	1,000,000	10,000,000	15,000	10,000	100,000	1,000
May 24.....	2	1,000,000,000	1,000,000,000	50,000,000	1,000	100,000	10,000
May 25.....	3	900,000,000	100,000,000	100,000,000	10,000	1,000	100
May 26.....	4	500,000,000	1,000,000,000	300,000,000	10,000	0	0
May 29.....	7	100,000,000	10,000,000	120,000,000	100,000	0	0
June 1.....	10	14,000,000	1,000,000,000	30,000,000	10,000	0	0
June 5.....	14	11,000,000	10,000,000	3,000,000	100,000	0	0
June 12.....	21	50,000,000	10,000,000	2,000,000	10,000	0	100
June 18.....	27	200,000	100,000	400,000	100,000	0	1,000
July 2.....	41	500,000	100,000	30,000	10,000	0	1,000
August 6...	76	1,000,000	100,000	700,000	10,000	0	0
August 28..	98	3,000,000	1,000,000	9,000,000	10,000	0	0
October 16..	147	50,000	0	100	10,000	0	0
December 20	212	300,000	100	20,000	1,000	0	0

ALFALFA AND CORN CHOP

May 24.....	0	2,000,000	100,000	150,000	100,000	100,000	10,000
May 25.....	1	1,000,000,000	1,000,000,000	20,000,000	10,000	1,000,000,000	1,000,000
May 26.....	2	2,000,000,000	100,000,000	200,000,000	10,000,000	10,000	10,000
May 29.....	5	520,000,000	1,000,000,000	400,000,000	1,000	1,000	1,000
June 1.....	8	250,000,000	10,000,000,000	100,000,000	10,000	0	100
June 5.....	12	120,000,000	10,000,000,000	120,000,000	10,000	0	100
June 12.....	19	140,000,000	100,000,000	20,000,000	10,000	100	0
June 18.....	25	16,000,000	10,000,000	40,000,000	1,000	0	100
July 2.....	39	250,000,000	10,000,000	14,000,000	10,000	0	0
August 6...	74	1,000,000	1,000,000	3,000,000	10,000	1,000	1,000
August 28..	96	10,000,000	100,000	90,000	100,000	0	0
October 16..	145	800,000	1,000	80,000	.....	0	10,000
December 20	210	15,000,000	100,000	1,000,000	100,000	0	0

ALFALFA AND SWEET-SORGHUM STOVER

May 31.....	0	120,000,000	10,000,000	70,000	10,000	10,000	1,000,000
June 1.....	1	200,000,000	100,000,000	10,000,000	10,000	100,000	10,000,000
June 2.....	2	500,000,000	1,000,000,000	600,000,000	1,000,000	10,000,000	10,000,000
June 4.....	4	2,000,000,000	1,000,000,000	10,000	10,000,000	1,000,000	1,000,000
June 7.....	7	1,000,000,000	100,000,000	500,000,000	1,000	10,000	10,000
June 9.....	9	600,000,000	100,000,000	250,000,000	10,000	100,000	10,000
June 11.....	11	240,000,000	100,000,000	100,000,000	100,000	0	100
June 14.....	14	250,000,000	10,000,000	50,000,000	100,000	1,000	10,000
June 18.....	18	50,000,000	10,000,000	25,000,000	10,000,000	1,000	10,000
July 2.....	32	60,000,000	10,000,000	15,000,000	1,000,000	10,000	10,000
August 6...	67	15,000,000	1,000,000	700,000	100,000	0	1,000
August 28..	89	120,000,000	1,000,000	30,000,000	10,000	10,000	10,000
October 16..	138	5,000,000	100,000	200,000	100	10,000	1,000
December 20	203	20,000,000	100,000,000	100,000	500,000	100,000	100,000

TABLE II.—*Microbial content of various kinds of silage—Experiments of 1915*—Contd.

ALFALFA AND RYE

Date.	Days.	Number of microorganisms per gram of silage.					
		Total.	Total acid producers.	Bulgarian group.	Gelatin liquefiers.	Colon type.	Yeasts.
June 1.....	0	50,000,000	1,000,000	210,000	10,000	100,000	100,000
June 2.....	1	400,000,000	100,000,000	40,000,000	10,000	10,000,000	10,000,000
June 4.....	3	1,000,000,000	1,000,000,000	1,000,000,000	10,000	1,000,000	1,000,000
June 7.....	6	300,000,000	10,000,000,000	270,000,000	10,000	1,000	0
June 9.....	8	60,000,000	100,000,000	50,000,000	100,000	0	0
June 11.....	10	240,000,000	100,000,000	60,000,000	10,000	100	0
June 14.....	13	45,000,000	10,000,000	10,000,000	100,000	100	0
June 18.....	17	20,000,000	10,000,000	10,000,000	1,000	10	0
July 2.....	31	15,000,000	100,000,000	5,000,000	10,000	0	1,000
August 6...	66	10,000,000	1,000,000	1,000,000	100,000	0	1,000
August 28..	88	2,000,000	1,000,000	100,000	1,000	100	100
October 16..	137	3,000,000	1,000,000	250,000	10,000	0	0
December 20	202	2,000,000	1,000,000	200,000	100,000	100	1,000

RYE

June 1.....	0	30,000,000	10,000,000	20,000	10,000	1,000	100,000
June 2.....	1	160,000,000	100,000,000	3,000,000	10,000	1,000,000	10,000,000
June 4.....	3	1,000,000,000	100,000,000	900,000,000	1,000	10,000,000	10,000,000
June 7.....	6	600,000,000	10,000,000,000	640,000,000	10,000	100	0
June 9.....	8	80,000,000	100,000,000	80,000,000	1,000	0	0
June 11.....	10	70,000,000	100,000,000	70,000,000	10,000	0	0
June 14.....	13	20,000,000	10,000,000	20,000,000	.....	0	0
June 18.....	17	7,000,000	1,000,000	3,000,000	100,000	0	100
July 2.....	31	15,000,000	1,000,000,000	5,000,000	1,000	0	10,000
August 6...	66	400,000	10,000	500,000	100,000	0	0
August 28..	88	100,000,000	10,000,000	14,000,000	10,000	100	10,000
October 16..	137	20,000,000	1,000,000,000	600,000	1,000	1,000,000	1,000,000
December 20	202	20,000,000	100,000	800,000	1,000,000	100,000	100,000

The microbial curve of development is similar to that noted in 1914, but is more pronounced and conforms more nearly to the results one may expect from normal-silage fermentation. The variations which occurred are attributed to the experimental error of the method of sampling.

No apparent differences were found that will account for the variations in the quality of silage. Stained preparations, made direct from the silage, appeared to agree with the cultural findings. In fact, if the smears were used as a basis of judging the quality of the silage, all would be considered high grade.

The offensive odors characteristic of the alfalfa silage is attributed to protein digestion. The number of gelatin liquefiers were determined in in each silo, with the hope of showing a possible difference between the types of silage. Some of the silage exhibited a slight increase in gelatin liquefiers, but this appears to be of little importance. In all probability the majority of liquefiers are spore-producing types which find unfavorable conditions for growth in the silage. Their numbers in all the silage remained fairly constant throughout the experiment. The slight increase noted in some cases is attributed to experimental error rather than to any actual increase of this type of organism.

The results obtained from these two years' investigations indicate that alfalfa when siloed alone undergoes a typical silage fermentation but that the final product is of a very poor quality. The fermentation, so far as has been observed, is caused by a microbial flora, practically identical with that obtained from silage made from the common forage crops.

However, when a fermentable carbohydrate supplement, such as molasses, corn chop, or sweet sorghum is added to alfalfa at time of siloing, a good quality of silage is produced. Little difference was noted between the microbial flora of such silage and that made from alfalfa alone. The chemical results indicate that more acid is produced in the silage containing the carbohydrate supplements than in that from alfalfa alone. The chemical and bacteriological data fail to offer sufficient evidence to account for the physical differences noted between the two types of silage.

The offensive odors noted in the alfalfa silage are no doubt the result of protein decomposition. By the addition of available carbohydrates this decomposition is checked, and a good quality of silage is the result.

EFFECT OF CARBOHYDRATES ON THE QUALITY OF ALFALFA SILAGE

Lipman and his associates (*13*, *14*) have demonstrated that utilizable carbohydrates when added to soil will hinder ammonification. Kendall and his coworkers (*7–10*), in studying the metabolism of bacteria, have likewise noted the protein-sparing effect of carbohydrates. That fermentation precedes putrefaction, when organisms are grown in media containing both protein and carbohydrates, has also been observed by Kligler (*11*) and Waksman (*18*).

Experiments were conducted for the purpose of observing under laboratory conditions the effect of carbohydrates upon alfalfa-silage fermentation.

Green and cured alfalfa was siloed in sterile milk bottles in the laboratory, with and without carbohydrates. The bottles were hermetically sealed and placed in the dark at room temperature. In those cases where cured alfalfa was used sufficient water was added to make up the proper moisture content. Upon opening the bottles the entire contents of each were finely ground by running through a sterile meat grinder, care being taken to handle as aseptically as possible.

Twenty gm. of this ground forage were placed in a 200–cc. sterile water blank, and from this the required dilutions for the bacteriological analysis were made.

For the chemical analysis 100 gm. of the ground forage were shaken in 1,000 cc. of distilled water for one hour. This was filtered, and an aliquot part of the filtrate used for the various chemical examinations. Twenty gm. of the ground forage were used for moisture determination.

The bacteriological analysis varied slightly in the different experiments, but the technic employed is the same as reported in the previous work. Except in some cases, casein agar was used for the determination of casein digesters, and all gelatin cultures were incubated for 10 instead of 5 days. The chemical analyses included moisture, acidity, amino nitrogen, and ammonia determinations. The acidity readings are expressed in number of cubic centimeters of *N/20* sodium hydroxid required to neutralize 100 gm. of silage. The amino nitrogen was determined by Van Slyke's method, and recorded as milligrams per 100 gm. of silage. The ammonia was determined by direct colorimetric readings of extracts of the ground forage and recorded as milligrams per 100 gm. of silage. All data are calculated upon a moisture-free basis. Three series of experiments were conducted, the first beginning on June 8, 1917, the second on June 25, and the third on July 10.

In the first series 15 milk bottles were filled with alfalfa and 15 with alfalfa plus 5 per cent of cane sugar added as a supplement. The first cutting of alfalfa was used in both cases. The results are given in Table III, first series.

The following notes pertaining to the aroma of the silage were recorded as the bottles were opened for analyses:

June 13. Alfalfa alone, odor good; alfalfa and cane sugar, odor good.
June 15. Alfalfa alone, odor good; alfalfa and cane sugar, odor good.
June 18. Alfalfa alone, odor not so good; alfalfa and cane sugar, odor good.
June 20. Alfalfa alone, odor not so good; alfalfa and cane sugar, odor good.
June 22. Alfalfa alone, undesirable odor; alfalfa and cane sugar, odor good.
September 5. Alfalfa alone, undesirable odor; alfalfa and cane sugar, odor good.
November 20. Alfalfa alone, undesirable odor; alfalfa and cane sugar, odor good.

The alfalfa silage at the conclusion of the experiment had the characteristic offensive odor which had been observed from the alfalfa silage made in the wooden silos. The alfalfa to which 5 per cent of cane sugar had been added produced a good quality of silage with a pleasant sour aroma.

The data submitted in Table III, first series, demonstrate that—

(1) There was little difference in the microbial flora of the two types of sileage.

(2) Both types exhibited a normal microbial curve of development—namely, a rapid increase of numbers for the first week, followed by a decrease.

(3) The acidophylic organisms are the predominating group.

(4) The gelatin liquefiers were inactive in the fermentation.

(5) The acidity produced was characteristic of alfalfa silage. The silage produced from alfalfa with a carbohydrate supplement contained a higher acid content than the alfalfa alone.

TABLE III.—*Effect of carbohydrate upon fermentation of alfalfa silage*

FIRST SERIES, JUNE 8

Days.	Silage.	Bacteriological analysis.						Chemical analysis.		
		Total number of bacteria per gram.	Number of Bulgarian type per gram.	Number of casein digesters per gram.	Number of total acid producers per gram.	Number of gelatin liquefiers per gram.	Percentage of moisture.	Quantity of *N*/20 sodium hydroxid per 100 gm. of silage.	Quantity of amino nitrogen per 100 gm. of silage.	Quantity of ammonia per 100 gm. of silage.
								cc.	*Mgm.*	*Mgm.*
0	Alfalfa alone	225,000,000	10,000	.....	1,000,000	1,000,000	73.5	236.17	197.43	.....
0	Alfalfa and sugar	3,000,000	10,000	.....	10,000,000	500,000	69.5	235.29	207.49	.....
3	Alfalfa alone	3,000,000,000	160,000,000	.....	1,000,000,000	10,000	66.6	725.22	358.17	.....
3	Alfalfa and sugar	2,500,000,000	1,300,000,000	.....	100,000,000	50,000	70.0	684.80	338.12	.....
5	Alfalfa alone	3,000,000,000	1,500,000,000	.....	1,000,000,000	50,000	74.0	730.70	367.30	.....
5	Alfalfa and sugar	2,000,000,000	1,000,000,000	.....	1,000,000,000	1,000	72.5	793.65	360.19	.....
7	Alfalfa alone	2,000,000,000	600,000,000	.....	10,000,000,000	1,000	75.0	752.70	367.65	.....
7	Alfalfa and sugar	3,000,000,000	2,000,000,000	.....	10,000,000,000	100	72.5	858.00	303.37	.....
10	Alfalfa alone	700,000,000	500,000,000	.....	1,000,000,000	1,000	72.5	686.40	405.21	.....
10	Alfalfa and sugar	250,000,000	300,000,000	.....	1,000,000,000	1,000	70.0	898.80	319.93	.....
13	Alfalfa alone	400,000,000	500,000,000	.....	10,000,000	1,000	72.5	643.50	415.93	.....
13	Alfalfa and sugar	400,000,000	200,000,000	.....	100,000,000	1,000	70.0	1,005.80	346.68	.....
14	Alfalfa alone	225,000,000	7,000,000	.....	50,000,000	1,000	75.0	731.00	442.90	.....
14	Alfalfa and sugar	200,000,000	14,000,000	.....	.....	1,000	70.0	920.20	326.23	.....
25	Alfalfa alone	75,000,000	25,000,000	.....	10,000,000	1,000	75.0	688.00	466.55	.....
25	Alfalfa and sugar	3,000,000	1,000,000	.....	1,000,000	1,000	72.5	922.35	351.52	.....
35	Alfalfa alone	160,000,000	130,000,000	.....	10,000,000	1,000	75.0	1,112.80	540.72	.....
35	Alfalfa and sugar	100,000	10,000	.....	10,000	1,000	72.5	1,201.28	383.77	.....
54	Alfalfa alone	12,000,000	200,000	.....	1,000,000	100,000	70.0	1,003.10	503.97	.....
54	Alfalfa and sugar	100,000	100	.....	10,000	10,000	80.0	1,166.40	368.28	.....
89	Alfalfa alone	20,000,000	8,000,000	.....	10,000,000	.....	77.5	711.50	546.72	.....
89	Alfalfa and sugar	5,000	2,000,000	.....	10,000,000	.....	67.5	918.00	362.32	.....
165	Alfalfa alone	30,000,000	1,000,000	.....	.....	1,000	75.0	602.00	516.64	.....
165	Alfalfa and sugar	80,000	2,000,000	.....	.....	1,000	70.0	898.80	399.96	.....

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SECOND SERIES, JUNE 25

0	Alfalfa alone	3,000,000	.....	.....	50,000	10,000	72.5	257.40	199.35	.....
0	Alfalfa and sugar	700,000	.....	.....	1,000,000	10,000	62.5	297.50	201.78	.....
2	Alfalfa alone	400,000,000	50,000,000	.....	1,000,000	100	70.0	509.60	292.11	.....
2	Alfalfa and sugar	800,000,000	70,000,000	.....	10,000,000,000	1,000	62.5	552.50	243.19	.....
4	Alfalfa alone	300,000,000	200,000,000	.....	10,000,000	100	72.5	622.00	250.74	.....
4	Alfalfa and sugar	300,000,000	200,000,000	.....	100,000,000	1,000	70.0	813.20	242.89	.....
7	Alfalfa alone	200,000,000	200,000,000	.....	100,000,000	100	70.0	535.00	348.82	.....
7	Alfalfa and sugar	100,000,000	23,000,000	.....	10,000,000	1,000	70.0	684.80	280.34	.....
11	Alfalfa alone	35,000,000	18,000,000	.....	100,000,000	100	66.0	747.20	328.32	.....
11	Alfalfa and sugar	30,000,000	8,000,000	.....	1,000,000,000	100	75.0	1,010.00	281.65	.....
14	Alfalfa alone	10,000,000	40,000,000	.....	1,000,000,000	100	70.0	834.60	323.14	.....
14	Alfalfa and sugar	40,000,000	20,000,000	.....	100,000,000	100	67.5	854.00	273.15	.....
26	Alfalfa alone	25,000,000	5,000,000	.....	1,000,000	1,000	68.0	743.00	422.76	.....
26	Alfalfa and sugar	2,000,000	70,000	.....	100,000	1,000	68.0	840.80	318.26	.....
72	Alfalfa alone	100,000	1,000	.....	10,000	.....	60.0	678.40	368.90	.....
72	Alfalfa and sugar	30,000	1,000	.....	50,000	.....	55.0	822.90	296.69	.....
148	Alfalfa alone	80,000,000	400,000	.....	.....	1,000	75.0	430.00	403.70	.....
148	Alfalfa and sugar	20,000	20,000	.....	.....	10,000	70.0	749.00	315.72	.....

THIRD SERIES, JULY 10

0	Alfalfa alone	60,000,000	20,000	4,000,000	1,000,000	100,000	72.5	345.00	164.01	22.41
0	Alfalfa and sugar	.....	17,000	2,000,000	10,000,000	10,000	72.5	.....	158.65	34.64
2	Alfalfa alone	3,000,000,000	500,000,000	.....	1,000,000,000	100,000	72.5	664.90	279.78	87.45
2	Alfalfa and sugar	.....	80,000,000	150,000	100,000,000	100,000	72.5	825.10	225.12	69.92
6	Alfalfa alone	400,000,000	7,000,000	3,000,000	10,000,000	100,000	74.0	730.30	369.45	147.13
6	Alfalfa and sugar	800,000,000	.....	.....	100,000,000	1,000	69.0	962.10	295.04	83.27
8	Alfalfa alone	800,000,000	100,000,000	2,000,000	100,000,000	100,000	77.5	689.60	398.49	112.06
8	Alfalfa and sugar	1,000,000,000	30,000,000	25,000	10,000,000	1,000	70.0	836.60	345.61	84.42
16	Alfalfa alone	75,000,000	5,000,000	15,000	10,000,000	100,000	72.5	750.70	485.61	263.83
16	Alfalfa and sugar	75,000,000	15,000,000	100,000	10,000,000	10,000	70.0	877.40	352.03	88.38
32	Alfalfa alone	30,000,000	50,000	20,000	1,000,000	100,000	75.0	838.50	.....	403.12
32	Alfalfa and sugar	3,000,000	1,000	3,000	10,000	10,000	70.0	856.00	.....	200.09
57	Alfalfa alone	10,000,000	1,000,000	15,000	50,000	.....	75.0	645.00	525.35	387.00
57	Alfalfa and sugar	6,000,000	1,000,000	14,000	50,000	.....	75.0	817.00	350.62	226.82
134	Alfalfa alone	20,000,000	2,000,000	100,000	.....	500,000	78.0	388.08	491.19	269.50
134	Alfalfa and sugar	2,000,000	20,000,000	60,000	.....	50,000	75.0	860.00	379.07	123.62

(6) More amino nitrogen was formed in the alfalfa silage than in the alfalfa and carbohydrate silage. This difference is clearly shown in figure 1.

(7) Carbohydrate supplements exert a protein-sparing effect.

In the second series of experiments begun on June 25, 10 milk bottles were filled with cured alfalfa and the same number with cured alfalfa plus 5 per cent of cane sugar. The alfalfa used was of the first cutting, having been cut on June 8. The data are presented in Table III, second series.

In the second series it was observed that alfalfa alone did not produce undesirable odors as rapidly as in the first series. The offensive odor was in evidence, however, on September 4, 72 days after siloing. The alfalfa with the supplement had a good odor until the series was discontinued, November 20, a period of 148 days.

A study of the data in Table III, second series, reveals the same general conclusion as observed in the preceding experiment. Curves showing the amino-nitrogen determinations of this series are shown in figure 2.

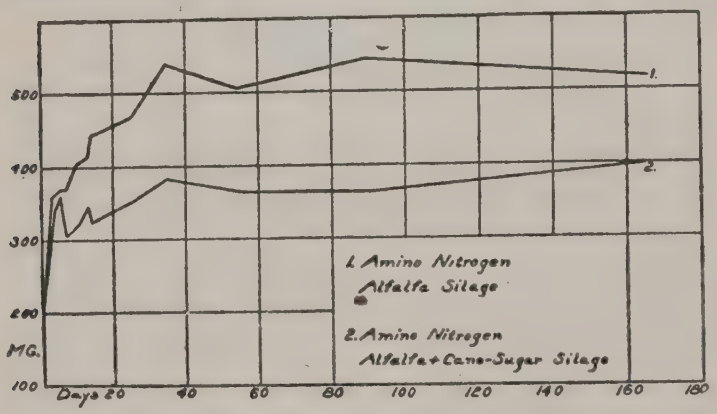

FIG. 1.—Graphs showing protein-sparing effect of carbohydrate in alfalfa-silage fermentation, first series.

The third series of experiments were conducted with the second cutting of alfalfa with and without cane sugar as in the previous experiments. The results are given in Table III, third series.

The quality of the silage as determined by odor was as follows:

July 16. Alfalfa alone, odor slightly undesirable; alfalfa and cane sugar, odor good.
July 26. Alfalfa alone, odor undesirable; alfalfa and cane sugar, odor good.
August 11. Alfalfa alone, odor undesirable; alfalfa and cane sugar, odor good.
September 5. Alfalfa alone, odor undesirable; alfalfa and cane sugar, odor good.
November 20. Alfalfa alone, odor very undesirable; alfalfa and cane sugar, odor good

The results presented in Table III, third series, are very similar to those of preceding experiments. In this series casein agar was used for the determination of casein digesters. The data obtained from this medium are in most respects very similar to those obtained from the use of gelatin. The ammonia determinations indicate the sparing effect of sugar upon the protein, as also do the amino-nitrogen determinations. The ammonia and amino-nitrogen determinations are plotted as curves in figure 3. They indicate the same general results as the curves plotted from the previous data.

It thus appears that physical and chemical differences between the two types of alfalfa silage were established. The data further demonstrate that the carbohydrate acts as a protein sparer.

The problem was discontinued at this point on account of the author's entering other work for the period of the war. As a result, the causative agents of the protein decomposition were not determined. However, the following suggestions pertaining to this question are offered:

It is recognized that the decomposition must be the result of microbial or plant-enzym activity or their associative actions. Previous publications issued from this laboratory mention the importance of microorganisms in silage fermentation (*5*, *6*). From these results it is concluded that the major fermentation processes in silage ripening are the result of microorganisms. That plant enzyms are active is not doubted, but their activities are of minor importance. Lamb in a recent paper on corn-silage fermentation (*12*) concludes (a) that bacteria are mainly responsible for the acid production and the disappearance of sugar; (b) that alcohol results from enzym activity first, but later by yeast fermentation; (c) that protein decomposition is caused by enzyms first, and later by microorganisms; and (d) that carbon dioxid is liberated by enzymic or respiratory changes primarily, but that yeasts are influential after the first day or two.

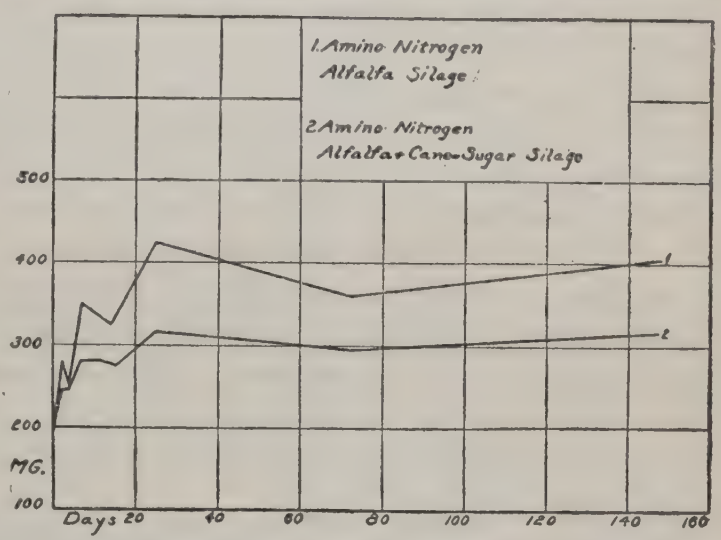

FIG. 2.—Graphs showing protein sparing effect of carbohydrate in alfalfa-silage fermentation, second series.

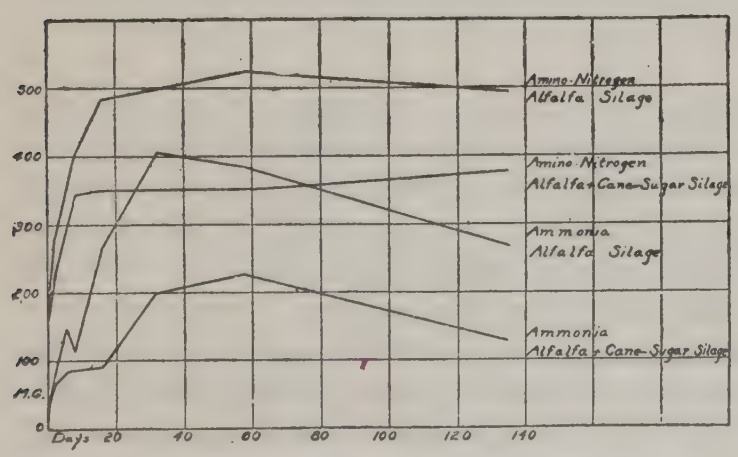

FIG. 3.—Graphs showing protein-sparing effect of carbohydrate in alfalfa-silage fermentation, third series.

Observations of silage made under laboratory conditions at this Station indicate that yeasts are the primary factors of alcohol and carbon-dioxid production. In the study of the microbial flora of silage it was noticed that as a rule the curve of yeast development indicated a very rapid increase of yeast cells within the first two or three days followed by a rapid to gradual decrease. It was further noticed that during the period between 12 and 72 hours after siloing the material underwent a violent fermentation, with the liberation of large amounts of carbon dioxid. During this early fermentation considerable

difficulty occurred in keeping the bottles sealed. This was finally accomplished by wiring the rubber stoppers and sealing with paraffin. That this fermentation was quite active was likewise noted by the bursting of several of the stoppered bottles during this stage. Forage siloed in the presence of chloroform failed to show this characteristic fermentation; nor did the material when opened indicate any signs of fermentation common to silage.

It is a natural assumption that yeasts would reach their maximum numbers and thereby cause an active fermentation in the early period of siloing. This active growth continues until the free oxygen has been consumed. At this stage their growth will be retarded somewhat by the anaerobic conditions produced, and finally checked by the acid fermentation. As a result of this yeast fermentation more or less alcohol is produced, but on account of the presence of the oxygen incorporated in the material when siloed, active growth development is stimulated probably more than alcoholic fermentation. The slow accumulation of alcohol may result from the small number of yeast cells, which persist in the silage for some time, or to zymase liberated from the degenerated yeast cells, or from plant enzyms.

Sherman and Bechdel (*16*), in a late publication pretaining to cornstover silage give as their opinion that the rôle of microorganisms is not as important as that of plant cells. The data offered are too meager and of such a character as not to justify their conclusion.

It is entirely plausible that plant enzyms may cause the protein decomposition noticed in the alfalfa silage. In view of the fact, however, that no such decomposition was observed in alfalfa siloed with chloroform and that chloroform is supposed to offer little injurious action upon such types of proteolytic enzyms, it appears that the cause it to be looked for elsewhere.

It is suggested that at least a small part of this proteolytic action may result from the ability of the acid producers to utilize protein as a source of energy in the absence of available carbohydrates. This digestive effect of the lactic acid bacilli has been demonstrated by Bertrand and Weisweiller (*1*), and Heinemann and Hefferan (*4*). Likewise, Hastings and his coworkers (*3*) show that from the analyses of pure lactic acid milk cultures, the percentage increase of soluble nitrogen varied from 12.5 to 62.5. Hart, Hastings, Flint, and Evans (*2*) have demonstrated the ability of *Bacillus casei* to produce ammonia. Hastings found large numbers of the *B. bulgaricus* group in chedder cheese, and concluded that, since they develop after the fermentation of the sugar, they must have some other source of carbon and energy than milk sugar.

No study of the anaerobic flora of the silage was made, but it would not be surprising to establish some proteolytic action with an anaerobic flora.

CONCLUSIONS

(1) Silage made from alfalfa alone is of an inferior quality. By the addition of an available carbohydrate supplement, a good quality of silage may be produced.

(2) The microbial flora of the two types of silage, so far as noted, was practically identical.

(3) The chemical data demonstrate that alfalfa with a supplement produced a higher acid content than alfalfa alone.

(4) Alfalfa silage shows a greater amount of protein decomposition, as evidenced by the amino-nitrogen and ammonia determinations.

(5) The decomposition of protein was checked by the addition of a utilizable carbohydrate.

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BROWN CANKER OF ROSES, CAUSED BY DIAPORTHE UMBRINA

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INTRODUCTION

In April, 1917, an apparently unreported fungus disease producing a canker on stems of rose (*Rosa* spp.) was brought to the attention of the Office of Pathological Collections. In the National Rose Test Garden at the Arlington Experimental Farm, Virginia, where over 900 named species and horticultural varieties of roses are grown, nearly all were affected. All the brier roses, the rugosas, the moss roses, some of the ramblers, and most of the named species were unaffected. The canes had been badly injured the previous season by a severe hailstorm, and the disease in some cases had gained entrance through these wounds, although there were many instances of infection where external injury could not be detected. The cankered canes were cut out and burned, and to all appearances the new growth was healthy. When observed in March, 1918, although there was no evidence of hail injury, the canker was again equally as prevalent as in 1917, thereby necessitating the severe cutting back of the plants.

The disease appears to be more or less widely distributed, specimens having been received from Georgia, Virginia, West Virginia, and the District of Columbia. The dates of these collections range from 1903 to 1918. In June, 1918, Dr. L. M. Massey, Plant Pathologist at Cornell University, who has given special attention to rose diseases, forwarded to this laboratory a culture made from rose material collected in Connecticut. A comparison of this culture with the fungus isolated by the writer showed that the two organisms are identical

CHARACTER OF THE DISEASE

This canker may occur on any portion of the cane, diseased areas being raw-umber in color, sometimes surrounded by a purple border (Pl. 46, B). The cankered surface is dotted with fruiting bodies, the pycnidial and ascosporic stages of the parasite. In mature specimens the small protruding beaks of the perithecia may be seen. The margin of a canker is generally defined only by the difference in color between the normal and cankered portions, and to this contrast in color is due the conspicuous and disfiguring effect of the disease (Pl. D). The canker differs in color and appearance from the common canker caused by *Coniothyrium fuckelii* Sacc. In color the latter canker is a Roman sepia or dirty

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umber. Its lesions are often depressed with raised margins, and the pycnidia in macroscopical appearance are darker than the fruiting bodies of the brown canker (Pl. 46, A).

ISOLATION OF THE CAUSAL ORGANISM

Following Keitt's method[1] for obtaining pure-culture strains, the writer made single pycnospore and single ascus isolations. It was thought advisable to isolate a single ascus rather than an ascospore, as the ascospores, especially when immature, appear much the same in in size and color as the pycnospores. From these isolations of the fungus transfers were made to various media. From the imperfect-stage strain only pycnidia developed, while from the perfect-stage strain growing on sterilized rose canes the formation of pycnidia was followed by the development of a perfect stage similar to that found in nature. The growth from the pycnidial stage was labeled "A," and that from the perithecial stage "B." Cultures of the two stages were used in inoculation experiments.

INOCULATION EXPERIMENTS

Inoculation tests were made on rose plants under ordinary greenhouse conditions and on cut rose canes kept in a moist atmosphere under bell jars in the laboratory. The inoculations were made by smearing spores or a portion of the culture over an incision in the cane made with a sterile scalpel. The incision was then covered with sterile cotton moistened with sterile water.

Inoculations with stage A in the greenhouse produced practically no infection. On one plant, which was inoculated on May 28, 1917, and placed under a bell jar, and which had gradually lost most of its leaves from mildew, the disease developed after a long period, producing the pycnidial stage of the fungus. Freshly cut rose canes placed under bell jars became infected in a few days. On stems inoculated on February 8 the disease had progressed upward 4 inches by February 20; and by February 23 it had progressed to the tip of the cane and had passed downward from the point of inoculation a distance of 4 inches, darkening and killing the stem. The cane was somewhat lighter in color for about 1 inch above and ½ inch below the point of inoculation. In this region were developed the characteristic pycnidia from which spore tendrils were exuded. The controls subjected to similar treatment remained fresh and healthy, and there was no discoloration of the tissue near the point of inoculation.

Inoculations were made with B in the greenhouse on October 20. In four days there was slight infection. The plants were kept under observation until January, but the area of infection remained very

[1] KEITT, G. W. SIMPLE TECHNIQUE FOR ISOLATING SINGLE-SPORE STRAINS OF CERTAIN TYPES OF FUNGI. *In* Phytopathology, v. 5, no. 5, p. 266–269, 1 fig. 1915.

limited, the cane being darkened and killed for a radius of about ¼ inch from the point of inoculation. A microscopic examination revealed the presence of hyphæ in the darkened tissue, but the fungus did not fruit on the diseased area. Plantings were made in corn-meal agar poured plates from small portions cut from the edge of the discolored tissue with a sterile scalpel, then immersed in a mercuric-chlorid solution (1:1,000) for about three minutes, and rinsed in sterile water. In such cultures made on January 19 the imperfect stage developed in five days, while cultures made similarly from the controls remained sterile.

Inoculations with B were again made in the greenhouse on January 30. No infection resulted from two of the inoculations, which may not have been kept sufficiently moist, but on February 13 it was observed that the third inoculation had produced infection. Plantings were made in April from the discolored region and from a corresponding region of the control. The fungus was reisolated from the inoculated plant, while no growth appeared in the culture made from the control (Pl. 47, C, D).

On January 17 freshly cut rose canes were placed under the bell jars in the laboratory and inoculated with B. Infection appeared in 10 to 15 days from all of the inoculations. The progress of the disease may be described from the observations taken on one of the inoculated canes, which when first observed was girdled and darkened for 1 inch above the point of inoculation. In two days the disease advanced upward 3 inches, and in three days the entire cane above the point of inoculation was affected. The disease passed downward less rapidly. An area, lighter in color and similar to that described in the A inoculations, appeared near the point of inoculation. In this area the typical pycnidia developed (Pl. 47, A, B).

Two inoculations were made by smearing an infusion of spores of stage B on the leaf buds. From each inoculation infection appeared at the base of the bud in about 10 days, and the disease advanced for several inches along the stem. The spores evidently germinated on the leaf tissue, the fungus passing through the bud into the cane. This experiment does not indicate that the fungus may gain entrance through the healthy or uninjured buds, for in making the inoculations no precaution was taken to avoid injuring the tender leaflets.

These experiments [1] establish the pathogenicity of the fungus. The percentage of infection appears to vary with temperature and humidity as shown by the results of the different series of inoculations. Inoculations made under bell jars gave 100 per cent infection, while those made without covering the plants gave a smaller percentage of infection. When the fungus is once established, it advances rapidly, producing the characteristic lesions.

[1] Subsequent to the writing of this paper, the information has been received from Dr. L. M. Massey, of Cornell University, that he has also made successful inoculations on roses in the greenhouse with what appears to be the same fungus.

DESCRIPTION OF THE FUNGUS

From a study of the fungi reported on the rose no description has been found that seems to apply to this organism. The characters of the ascogenous stage, both in its development in nature and in culture, place it most nearly in the genus Diaporthe. It differs from that genus in having continuous ascospores which may, however, become pseudo-septate. The spores of the genus Diaporthe are typically 2-celled, but in some species they are described as nucleate, 1-celled while young, or obscurely septate. The imperfect stage appears to be most closely related to the genus Phomopsis. In view of the above considerations, it has seemed that for the present the fungus may best be referred to the genus Diaporthe and will be described as *Diaporthe umbrina*.

Diaporthe umbrina, n. sp.[1]

Pycnidia subglobose to lens-shaped, walls generally rather thin, thickened at apex, embedded, rupturing the epidermis, irregularly ostiolate, simple or chambered, 200 to 300 μ in diameter; pycnospores subfusoid, straight or slightly curved, hyalin, 4.8 to 11.2 by 2 to 3.2 μ; sporophores simple or branched, tapering above, 12 to 40 μ in length.

Perithecia membranaceous, two to five, immersed in a valsoid stroma around pycnidium, globose, with beak scarcely projecting above the epidermis, 100 to 290 μ in diameter; beaks 150 to 195 μ in length; asci clavate, subsessile, aparaphysate, 30 to 50 by 6.4 to 8 μ; spores elliptical, usually hyalin, when mature sometimes light olivaceous, continuous or sometimes with one pseudo-septum, 8 to 11.2 by 3.2 to 4 μ.

Forms cankers on the stems of cultivated roses.

Type material collected at Arlington Experimental Farm, Virginia, April, 1917. Specimens deposited in the herbarium of the Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

In figure 1 is shown a pycnidium as it occurs in nature. The basal wall of the pycnidium is often very much thickened near the center, projecting upward in the cavity of the pycnidium. Protrusions may develop from other portions of the wall, forming a chambered pycnidium as illustrated in figure 1, *a*. The parenchymatous upper portion of the pycnidium is composed of small, closely packed, thin-walled cells. The fungus suggests somewhat the appearance of a species of Myxosporium in certain sections through the pycnidium in which the thickened upper portion has apparently fallen away. Great variability was apparent in the development of the pycnidial stage in culture. It was observed that on prune-agar poured plates made from stage A that the spores were first borne directly from the mycelium and that an abundance of

[1] **Diaporthe umbrina**, sp. nov.—Pycnidiis subglobosis vel lenticularibus, parietibus plerumque aliquid tenuibus, apice incrassatis, immersis, epidermidem rumpentibus, irregulariter ostiolatis, simplicibus vel locellatis, 200–300 μ diameter pycnosporis subfusoideis, rectis vel curvulis, hyalinis, 4.8–11.2×2–3.2 μ, sporophoris simplicibus vel ramosis, attenuatis, 12–40 μ longis; peritheciis membranaceis, 2–5, valsea stromate circa pycnidium immersis, globosis, rostratis, 100–290 μ diameter; rostris saepe supra epidermidem prominentibus, 150–195 μ longis; ascis clavatis, subsessilibus, aparaphysatis, 30–50×6.4–8 μ; sporis ellipticis, plerumque hyalinis, maturis interdum pallide olivaceis, continuis vel interdum 1-pseudo-septatis, 8–11.2×3.2–4 μ.

Cancros in sarmentis Rosarum cultarum formans, Arlington, Virginia.

thin-walled membranous pycnidia soon developed. The fungus was transferred to the rose-stem medium, where the pycnidial development was similar to that in nature, the pycnidia being simple or somewhat chambered, 140 to 375 μ in diameter with the walls in the upper portion somewhat more stromatic. The perithecia develop in the stromatic growth below the pycnidia which in culture become erumpent. Plate 46, C, shows an enlarged portion of a culture, with protruding beaks of the perithecia. In culture there may be as many as eight perithecia in a stroma, measuring 290 to 400 μ, with beaks 250 to 500 μ in length. Figure 2, *a*, shows a vertical section of perithecia in nature. It will be observed from this illustration that in nature the beaks of the perithecia which extend toward the pycnidial rupture in the epidermis do not protrude proportionally as far as they do in culture. In figure 3 is shown the arrangement of the perithecia and pycnidia as they occur in nature.

FIG. 1.—*Diaporthe umbrina: a*, Vertical section of a pycnidium in nature, × 80; *b*, simple and branched sporophores; *c*, pycnospores, × 360.

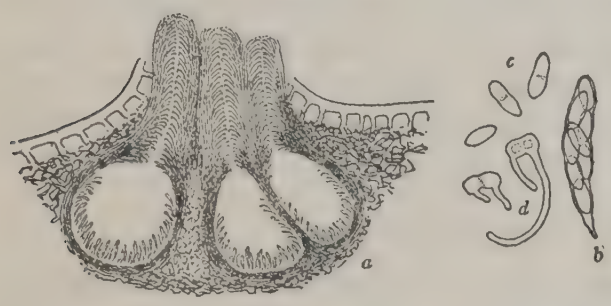

FIG. 2.—*Diaporthe umbrina: a*, Vertical section of perithecia in nature, × 80; *b*, an ascus; *c*, ascospores; *d*, germinating ascospores, × 420.

The development of pycnospores on the host and in culture is similar. The filiform spores which sometimes develop in species of Phomopsis in addition to the typical fusoid spores have not been observed. The pycnospores germinate readily, producing one or two germ tubes. The sporophores, which are sometimes branched, are variable in length, but generally measure from 12 to 20 μ. The base of the sporophore is often broad and irregular in shape, becoming very slender above (fig. 1, *b*). The ascospores in nature are most often continuous, but they may be 2-guttulate or 1-pseudoseptate. Upon germination the spores become 2-guttulate or pseudoseptate and may be slightly constricted at the center, and one germ tube is produced toward each end of the spore (fig. 2, *b*, *c*, *d*).

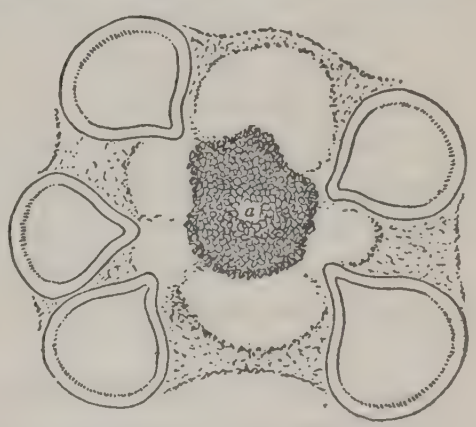

FIG. 3.—*Diaporthe umbrina:* Tangential section showing relative arrangement of pycnidium and perithecia in nature, a pycnidium surrounded by five perithecia; *a*, proliferous stratum extending upward from base of pycnidium.

CULTURAL CHARACTERS

The organism was grown on steamed corn meal, corn-meal agar, oat agar, prune agar, and rose canes. Both strains of the fungus grow rapidly on these media, producing the imperfect stage in three or four days with very little superficial mycelial growth. The pycnidia are developed in great abundance, extruding cinnamon-buff[1] spore masses, which often cover the entire surface of the medium.

Plate 46, D, shows a B culture on rose canes after 22 days' growth, while Plate 46, C and E, reproduces photomicrographs of a similar culture after three months, showing the extruding pycnospore masses and the beaks of the perithecia. Only cultures on rose stems in test-tubes containing a very small amount of moisture and kept approximately at a temperature of 17° C. developed the perfect stage of the fungus. On steamed corn-meal media the superficial mycelial growth was most abundant, appearing pure white at first and soon becoming gray. On prune agar and on corn-meal agar poured plates the culture develops radially from the point of inoculation, concentric rings of the pycnidia appearing at more or less regular intervals.

LIFE HISTORY OF THE FUNGUS

That the life cycle of the fungus may be completed in a comparatively short time is shown from the cultural experiments and from the field observations. From the inoculation experiments it has been shown that infection occurs from both pycnospores and ascospores and that lesions may be produced in from 4 to 15 days. A culture made on February 11 from pycnospores produced from stage B had developed the two stages by April 15. On field material collected on March 18 the imperfect stage was present, together with the immature perfect stage. In May spores of both stages germinated in culture, producing the perfect and imperfect stages as previously described, indicating that a period of rest is not essential for the completion of the life cycle. On the other hand, the spores probably remain viable for a long time, since pycnospores from very thin poured-plate cultures which had dried were viable after having been kept for four months at a temperature of about 17° C. How the fungus overwinters and the manner in which the first infections are produced are still subjects of investigation. It is very probable that the ascospores live over a winter in the old canker and produce the early spring infections. It is not known how the spores are disseminated, whether by wind, rain, insects, or on cultural implements, but it is probable that rain plays an important part in distributing the exuded pycnospores.

CONTROL MEASURES

Preliminary experiments in controlling the disease by cutting out and burning the cankered stems have been made, but the results during the

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

single year that the disease has been under observation were negative. From the present knowledge of this rose canker and its causal fungus, together with the control measures employed for diseases of a similar nature, certain preventive methods are suggested. Rose gardens should be carefully inspected to determine whether the disease is present and precautions taken to prevent the entrance of the fungus into healthy gardens. Nursery stock for planting should be carefully examined, and all cankered plants should be destroyed. If possible, plants should be introduced only from gardens known to be free from the disease. In gardens where the canker is already established, measures leading to the eradication of the fungus should be adopted. Cankered stems should be cut away and burned. It may be advisable to disinfect the cut ends of the stems and to sterilize the pruning implements after each operation. The use of a fungicide to protect the plants from further infection is suggested. In the fall after the plants have become dormant and again early in the spring strong Bordeaux mixture[1] may be applied. It is probably important that the plants be well covered with the fungicide during the early spring months when the lesions first appear and the disease makes rapid progress. Gardens should be carefully watched in the spring in order that stems showing infection may be cut away and not become sources of secondary infection. As blossoming time approaches, ammoniacal copper carbonate may be substituted for the Bordeaux mixture, as it does not discolor the foliage. A complete study of the varieties resistant to this canker has not been made, but, as stated in the introduction of this paper, in the National Rose Test Garden, where most classes of cultivated roses are represented, the brier roses, the rugosa roses, the moss roses, some of the ramblers, and most of the named species are apparently resistant.

SUMMARY

(1) A canker of roses caused by the fungus *Diaporthe umbrina* is probably widely distributed and is known to occur in the District of Columbia, Virginia, West Virginia, Georgia, and Connecticut, having been reported at various times during the past 15 years.

(2) The causal organism produces in cankers on living rose stems a pycnidial and a perfect stage.

(3) In culture both the pycnidial and perithecial stages of the fungus have developed.

(4) The disease has been produced on rose stems from both pycnosporic and ascosporic stages of the fungus.

(5) The control measures suggested are the use of only healthy nursery stock for planting, the removal and burning of diseased canes from affected gardens, and the application of a fungicide in the fall, again in spring before the first symptoms appear, and during the spring when the fungus is active.

[1] For directions for the preparation and application of fungicides for rose diseases see MULFORD, F. L., ROSES FOR THE HOME. U. S. Dept. Agr. Farmers' Bul. 750, 36 p., 27 fig. 1916.

PLATE D

Rose cane showing lesion of the canker caused by *Diaporthe umbrina*.

(600)

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PLATE 46

A.—Rose cane showing the appearance of the canker caused by *Coniothyrium fuckelii*.

B.—A rose stem showing local infections produced by *Diaporthe umbrina*.

C.—Culture of *Diaporthe umbrina* from stage B on a rose stem showing beaks of perithecia. × 20.

D.—Culture on a rose stem from stage B showing spore masses extruded from pycnidia.

E.—Pycnospore masses from culture shown in figure C. × 20.

Photographed by Mr. J. M. Shull.

A
B
C
D
E

A
B
C
D

PLATE 47

Diaporthe umbrina: Results of inoculations

A.—Control.

B.—Rose stem showing infection produced by inoculation with stage B; cut rose stems placed in moist atmosphere under bell jars in the laboratory.

C.—Control.

D.—Rose stem showing infection produced by inoculation with stage B; rose plants in the greenhouse.

Photographed by Mr. J. M. Shull.

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EFFECT OF CARBON DISULPHID AND TOLUOL UPON NITROGEN-FIXING AND NITRIFYING ORGANISMS

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INTRODUCTION

In previous publications [1] the writer has presented experimental data showing the effect which carbon disulphid and toluol have upon the total number of bacteria (bacteria that would develop upon agar plates) and upon the accumulation of nitrate nitrogen in soils. It is the purpose of this paper to give data showing the effect of similar treatment of soils upon the nitrogen-fixing organisms, together with additiomal data upon nitrification.

The appearance during the past few years of many excellent reviews of the literature relative to the treatment of soils with volatile antiseptics renders a discussion of previous work unnecessary. The reader is referred especially to the review given by Kopeloff and Coleman. [2] It is sufficient to say that the results heretofore reported have been very irregular and inconclusive. It is believed that the data reported in this paper will offer, in part at least, an explanation for some of these irregularities.

EXPERIMENTAL METHODS

In general, the methods used have been similar to those previously employed by the writer. Soils known to possess good nitrogen-fixing and nitrifying powers have been used. Given quantities of soil by weight were treated with varying quantities of carbon disulphid and with toluol. The reagent was then thoroughly mixed in, and the soil immediately placed in an air-tight container and left for three days. At the end of this period those samples from which the reagent was not to be evaporated were made up to the required degree of saturation with sterile water, the rubber stoppers replaced with cotton, and incubated at room temperature. If the antiseptic was to be evaporated, either the containers were left open or the soil was emptied into sterile petri dishes and left until the soil was air-dry and no odor of the chemical remained. Water was then added and the samples incubated at room temperature. At various stages during treatment or during incubation the nitrogen-fixing ability was tested, and the nitrates present in the soil were determined.

[1] GAINEY, P. L. THE EFFECT OF TOLUOL AND CS_2 UPON THE MICROFLORA AND FAUNA OF THE SOIL. *In* Mo. Bot. Gard. 23d Ann. Rpt., p. 147–169. 1912. Literature, p. 168–169.

——— EFFECT OF CS_2 AND TOLUOL UPON NITRIFICATION. *In* Centbl. Bakt. [etc.]. Abt. 2, Bd. 39, No. 23–25, p. 584–595, 2 fig. 1914.

[2] KOPELOFF, Nicholas, and COLEMAN, D. A. A REVIEW OF INVESTIGATIONS IN SOIL PROTOZOA AND SOIL STERILIZATION. *In* Soil. Sci., 3, no. 3, p. 197–269. Literature cited, p. 248–269.

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In the nitrogen-fixing experiments 50 cc. of a sterile culture solution in 350-cc. Erlenmeyer Jena flasks were inoculated with 2 or 5 gm. of soil and incubated, usually for three weeks. A medium consisting of the following compounds was used:

Potassium phosphate (Kn_2HPO_4)	0.2 gm.
Magnesium sulphate ($MgSO_4$)	.2 gm.
Sodium chlorid (NaCL)	.5 gm.
Mannit	20.0 gm.
Ferric chlorid ($FeCl_3$)	Trace.
Water	1,000 cc.

This solution was made slightly alkaline to phenolphthalein with sodium hydroxid. In other experiments this medium has never failed to give the characteristic Azotobacter film if the organisms were present in sufficient numbers and other conditions were favorable. The figures reported are in all cases actual gains in nitrogen.

Inoculations were made in duplicate. During incubation the growth of Azotobacter was noted, and at the end of the incubation period the total nitrogen was determined. Only the average of duplicate nitrogen determinations are reported.

In the tables giving results the Azotobacter film has been reported as typical if the characteristic brown or black uniform growth covered the surface. A question mark (?) has been placed opposite those samples from which only one duplicate gave a film or when there were Azotobacter present, but which failed to give the characteristic growth.

Nitrate nitrogen was determined in duplicate by the phenol-disulphonic-acid method. Qualitative tests for ammonia were made with Nessler's reagent. Where the aqueous extract of the soil gave only a slight yellow color, it was reported as a trace. When the color was somewhat more pronounced, it was reported as slight; and where a heavy brick-red precipitate was formed when the reagent was added, it was reported as abundant. In some instances quantitative determinations of ammonia nitrogen were made by distillation with magnesium oxid. A number of determinations upon samples reported as a trace showed from 0.3 to 0.5 mgm. of nitrogen per 100 gm. of soil. Variations from these methods were sometimes made, but they are mentioned in connection with the individual experiment to which they apply.

EXPERIMENTAL DATA

The soil for these experiments was furnished through the kindness of Prof. Walter G. Sackett, of the Colorado Agricultural Experiment Station. It came "from a locality where the niter trouble has been very severe and where nitrification has evidently been very active in the past if not at present." This soil was used because of the exceptionally vigorous nitrogen-fixing (Azotobacter) flora.

Fifty-gm. samples containing 6.5 gm. of water each were treated with varying amounts of toluol and carbon disulphid as indicated in Table I. The soil was then immediately placed in 500-cc. bottles, tightly stoppered, and left for three days. At the end of this time the water content of the nonevaporated samples was made up to 12 cc. or one-half saturation, and the stopper replaced with cotton. The samples were incubated under conditions which would retard loss of moisture and were not opened except when analyses were made. The evaporated samples were treated exactly alike, except that at the end of the first three days the soil was emptied into petri dishes, left thus for 48 hours, replaced in the bottle, and the moisture content made up to one-half saturation. Tests for nitrogen fixation were made at the end of four weeks and again after six months' incubation, and the nitrate content was determined at the end of six months.

Before the six-months' analyses were made the moisture content of all samples had fallen very low, some samples being practically air-dry. This apparently had no effect upon nitrogen-fixing organisms, but did obscure the recovery of active nitrification. In other words, before sufficient time had elapsed for the recovery of active nitrification, the moisture content of many samples had fallen so low that nitrification was impossible. The results are reported in Table I.

There is little difference between the evaporated and nonevaporated samples in the amount of nitrogen fixed; 0.25 cc. of toluol per 100 gm. of soil destroyed the Azotobacter, and there is no evidence of recovery. The same quantity checked nitrification, but the quantity necessary to destroy the nitrifying organisms is very much higher, the samples receiving even 5 cc. having partly recovered after six months. As mentioned above, the failure to recover was probably due to low water content rather than to the toluol. Ammonia accumulated only when nitrification was checked.

The quantity of carbon disulphid necessary to destroy Azotobacter was only 0.1 cc. per 100 gm. of soil, and there is no evidence of recovery even after six months. In the nonevaporated samples, 0.1 cc. checked nitrification, while 0.25 cc. were required to check the process in evaporated samples. There is evidence of recovery of nitrification even with 5.0 cc. of carbon disulphid. Ammonia accumulated only when nitrification was checked.

Even when Azotobacter were destroyed, the ability to fix nitrogen was not destroyed with the largest quantity of carbon disulphid or toluol applied in these experiments. However, fixation by organisms other than Azotobacter appeared to decrease as the application of chemicals increased.

A second set of experiments was conducted, in which the soil had been in the laboratory much longer and was almost air-dry, containing only

4 per cent of water. The treatment of the soil otherwise was essentially the same. After the reagents had acted for three days, the unevaporated samples were made up to one-half saturation. Those from which the reagents were evaporated were left open for two days and then made up to the same degree of saturation. All were cotton-plugged and incubated at room temperature. The water lost through evaporation was replaced from time to time.

TABLE I.—*Effect of carbon disulphid and toluol upon nitrogen fixation and nitrate accumulation*

CHEMICALS NOT EVAPORATED

Treatment.	Analyzed after four weeks.			Analyzed after six months.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3 [b]	Azotobacter film.	Nitrogen fixed.[a]	NO_3 [b]	NH_3 [b] nitrogen.
		Mgm.	*Mgm.*		*Mgm.*	*Mgm.*	
Control, 0 cc	Typical.	10. 40	11. 3	Typical.	9. 12	18. 5	Trace.
Toluol:							
0. 01 cc	...do....	11. 80	12. 0	...do....	9. 70	18. 5	Do.
0. 05 cc	...do....	12. 40	11. 3	...do....	9. 01	18. 5	Do.
0. 10 cc	...do....	11. 75	12. 0	...do....	9. 07	18. 5	Do.
0. 25 cc	None...	7. 05	4. 5	None....	4. 32	20. 0	Do.
0. 50 cc	...do....	10. 55	3. 0	...do....	3. 40	3. 5	Abundant.
1. 00 cc	...do....	7. 05	4. 5	...do....	4. 61	3. 5	Do.
5. 00 cc	...do....	7. 75	3. 6	...do....	5. 27	3. 3	Do.
Control, 0 cc	Typical.	12. 45	12. 8	Typical.	9. 12	18. 5	Trace.
Carbon disulphid:							
0. 01 cc	...do....	12. 05	12. 8	...do....	9. 18	18. 5	Do.
0. 05 cc	...do....	11. 90	12. 8	(?)	7. 45	18. 5	Do.
0. 10 cc	None....	4. 25	13. 8	None....	5. 32	20. 0	Do.
0. 25 cc	...do....	4. 45	3. 2	...do....	3. 13	4. 1	Abundant.
0. 50 cc	...do....	3. 45	2. 2	...do....	0. 55	3. 5	Do.
1. 00 cc	...do....	2. 15	3. 3	...do....	0. 65	3. 3	Do.
5. 00 cc	...do....	3. 15	3. 5	...do....	6. 68	3. 2	Do.

CHEMICALS EVAPORATED

Control 0 cc	Typical.	9. 45	12. 0	Typical.	10. 28	21. 8	Trace.
Toluol:							
0. 01 cc	...do....	9. 20	12. 0	...do....	9. 18	21. 8	Do.
0. 05 cc	...do....	8. 67	10. 9	...do....	10. 28	21. 8	Do.
0. 10 cc	...do....	10. 75	12. 0	...do....	9. 62	20. 0	Do.
0. 25 cc	None....	4. 05	3. 6	None....	5. 00	24. 0	Do.
0. 50 cc	...do....	5. 20	.....	...do....	5. 38	12. 0	Abundant.
1. 00 cc	...do....	5. 05	3. 6	...do....	4. 17	21. 8	Trace.
5. 00 cc	...do....	4. 45	3. 5	...do....	5. 27	6. 7	Abundant.
Control 0 cc	Typical.	10. 00	12. 0	Typical.	9. 73	19. 2	Trace.
Carbon disulphid:							
0. 01 cc	...do....	10. 10	12. 0	...do....	9. 73	24. 0	Do.
0. 05 cc	...do....	9. 35	12. 0	(?)	6. 87	21. 8	Do.
0. 10 cc	None....	4. 45	3. 5	None....	4. 12	21. 8	Do.
0. 25 cc	...do....	3. 35	3. 5	...do....	3. 35	6. 3	Abundant.
0. 50 cc	...do....	3. 30	3. 5	...do....	.....	5. 8	Do.
1. 00 cc	...do....	3. 15	3. 5	...do....	2. 74	4. 8	Do.
5. 00 cc	...do....	2. 80	2. 0	...do....	3. 24	12. 0	Do.

[a] Nitrogen fixed in milligrams per 50 cc. of culture. [b] Nitrate in milligrams per 100 gm. of soil.

Cultures for Azotobacter were made from the evaporated samples after the exposure for evaporation and from all cultures after 16 weeks' incubation. Since the principal point to be determined was whether Azotobacter were killed, a total nitrogen determination was not made on the nitrogen-fixing cultures at the 16 weeks' analysis. Also, previous experiments had shown that the quantity of nitrogen fixed when the characteristic film developed did not vary beyond the experimental error and that the quantity fixed in absence of Azotobacter was usually from one-fourth to one-half than when Azotobacter were present. The character of growth was, therefore, a sufficient index for the object in view. The results are presented in Tables II and III.

TABLE II.—*Effect of toluol upon nitrogen fixation and nitrification*

Treatment.	Toluol not evaporated.			Toluol evaporated.				
	Incubation 10 weeks.	Incubation 17 weeks.		No incubation.[a]	Incubation 10 weeks.	Incubation 17 weeks.		
	Azotobacter film.	NO_3.[a]	NH_3 nitrogen.	Azotobacter film.	Nitrogen fixed.[b]	Azotobacter film.	NO_3.[a]	NH_3 nitrogen.
		Mgm.			*Mgm.*		*Mgm.*	
Control, 0 cc....	Typical.	17.5	Trace.	Typical.	.....	Typical.	18.0	Trace.
Toluol:								
0.02 cc.....	...do....	18.5	...do..	...do....	8.23	...do....	16.4	Do.
0.10 cc.....	...do....	16.9	...do..	...do....	7.56	...do....	16.9	Do.
0.20 cc.....	...do....	16.4	...do..	...do....	8.34	...do....	18.5	Do.
0.40 cc.....	...do....	19.2	...do..	...do....	9.91	...do....	17.5	Do.
0.60 cc.....	...do....	16.2	...do..	...do....	8.51	...do....	.....	Do.
0.80 cc.....	...do....	16.2	...do..	...do....	8.28	...do....	16.9	Do.
1.00 cc.....	...do....	18.0	...do..	...do....	10.64	...do....	17.5	Do.
1.20 cc.....	...do....	18.0	...do..	...do....	9.30	...do....	17.5	Do.
1.40 cc.....	...do....	16.9	...do..	...do....	8.45	...do....	17.5	Do.
1.60 cc.....	...do....	20.7	...do..	...do....	9.13	...do....	18.0	Do.
1.80 cc.....	...do....	16.9	...do..	...do....	9.63	...do....	17.5	Do.
2.00 cc.....	...do....	19.2	...do..	...do....	8.40	...do....	16.9	Do.
10.00 cc.....	None....	5.8	[c] 3.0	...do....	9.02	...do....	18.0	Do.

[a] Nitrates in milligram per 100 gm. of soil.
[b] Nitrogen fixed in milligram per 50 cc. of culture.
[c] Milligrams.

The results presented in Tables II and III are quite different from those given in Table I. For example, the only samples in which Azotobacter were destroyed and in which nitrification was checked were those treated with 10 cc. of either carbon disulphid or toluol. In case of the evaporated samples, even 10 cc. of toluol had no effect. Again, in only those samples in which nitrification was checked was there any accumulation of ammonia.

TABLE III.—*Effect of carbon disulphid upon nitrogen fixation and nitrate accumulation*

Treatment.	Carbon disulphid not evaporated.		Carbon disulphid evaporated.					
	Incubation 16 weeks.	Incubation 17 weeks.	No incubation.			Incubation 16 weeks.	Incubation 17 weeks.	
	Azotobacter film.	NO_3.[a]	NH^3 nitrogen.	Azotobacter film.	Nitrogen fixed.[b]	Azotobacter film.	NO_3.[a]	NH_3 nitrogen.
		Mgm.			*Mgm.*		*Mgm.*	
Control, 0 cc....	Typical.	18.0	Trace.	Typical.	9.18	Typical.	17.0	Trace.
Carbon disulphid:								
0.02 cc.....	...do....	18.0	...do..	...do....	10.64	...do....	20.0	Do.
0.10 cc.....	...do....	18.0	...do..	...do....	.....	...do....	19.4	Do.
0.20 cc.....	...do....	18.0	...do..	...do....	9.13	...do....	19.7	Do.
0.40 cc.....	...do....	17.0	...do..	...do....	11.03	...do....	20.0	Do.
0.60 cc.....	...do....	18.0	...do..	...do....	.....	...do....	17.0	Do.
0.80 cc.....	...do....	17.0	...do..	...do....	8.25	...do....	18.0	Do.
1.00 cc.....	...do....	18.0	...do..	...do....	.....	...do....	18.5	Do.
1.20 cc.....	...do....	17.0	...do..	...do....	9.91	...do....	19.0	Do.
1.40 cc.....	...do....	17.5	...do..	...do....	8.56	...do....	21.1	Do.
1.60 cc.....	...do....	19.4	...do..	...do....	12.32	...do....	20.6	Do.
1.80 cc.....	...do....	19.0	...do..	...do....	8.45	...do....	17.0	Do.
2.00 cc.....	...do....	18.0	...do..	...do....	9.20	...do....	16.3	Do.
10.00 cc.....	None....	7.8	[c] 1.9	None....	−0.84	None....	6.6	[c] 2.0

[a] Nitrates in milligrams per 100 gm. of soil.
[b] Nitrogen fixed in milligrams per 50 cc. of culture.
[c] Milligrams.

EFFECT OF MOISTURE CONTENT OF SOIL UPON THE GERMICIDAL ACTION OF CARBON DISULPHID AND TOLUOL

The results obtained from the two sets of experiments given above were so radically different that it seemed necessary to ascertain, if possible, the cause of the differences. The soil, while not the same, was quite similar and it did not seem possible that such wide differences in the effect of the reagents could be due to differences in the character of the soil. An examination of the conditions under which the two sets of experiments were conducted revealed only one important difference. The moisture content of the soil in the first experiments was 13 per cent, while in the second set the moisture content was only 4 per cent. Experiments were therefore planned to throw some light upon the influence of the moisture content of soil on the effectiveness of carbon disulphid and toluol in killing Azotobacter.

In these experiments the same soil was employed as in the second set. Three conditions of the soil with respect to moisture were compared. In the first the water content was 3 per cent, in the second 10 per cent, and in the third 20 per cent. The soil was made up to different moisture contents before treatment.

After the chemical was allowed to act for three days all samples were cultured for Azotobacter. The bottles were then opened and left open

until the soil became approximately air-dry. This was two days for the first and second sets and six days for the third set. During this time, in order to increase the surface exposed to air and also prevent contamination, the bottles were left lying on their sides under a hood.

After exposing the samples for the chemical to evaporate, another series of cultures were made for Azotobacter. The water content of the soil was then made up to one-half saturation and incubated at room temperature. The water lost by evaporation was restored from time to time. At the end of three months' incubation a third series of cultures for Azotobacter were made and the nitrate content determined.

The actual quantities of nitrogen fixed were not determined, for reasons already given, the character of growth being considered a sufficient index. The results are presented in Table IV.

TABLE IV.—*Effect of carbon disulphid and toluol upon Azotobacter and nitrate accumulation in soil*

WATER CONTENT OF SOIL, 3 PER CENT

Treatment.	Azotobacter film.			NO_3[b].	NH_3.
	1[a]	2[a]	3[a]		
				Mgm.	
Control, 0 cc	Typical	Typical	Typical	13.2	Trace.
Do.	do.	do.	do.	12.0	Do.
Carbon disulphid:					
0.10 cc	do.	do.	do.	12.0	Do.
0.10 cc	do.	do.	do.	12.0	Do.
0.25 cc	do.	do.	do.	12.0	Do.
0.25 cc	do.	do.	do.	12.6	Do.
0.50 cc	do.	do.	do.	12.0	Do.
0.50 cc	do.	do.	do.	12.0	Do.
1.00 cc	do.	do.	do.	11.4	Do.
1.00 cc	do.	do.	do.	12.0	Do.
5.00 cc	do.	do.	do.	12.6	Do.
5.00 cc	do.	do.	do.	11.7	Do.
Toluol:					
0.10 cc	do.	do.	do.	12.6	Do.
0.10 cc	do.	do.	do.	12.6	Do.
0.25 cc	do.	do.	do.	12.0	Do.
0.25 cc	do.	do.	do.	11.7	Do.
0.50 cc	do.	do.	do.	10.0	Do.
0.50 cc	do.	do.	do.	11.0	Do.
1.00 cc	do.	do.	do.	12.0	Do.
1.00 cc	do.	do.	do.	12.6	Do.
5.00 cc	do.	do.	do.	12.4	Do.
5.00 cc	do.	do.	do.	12.6	Do.

[a] 1, 2, and 3 have reference to time at which cultures were made. 1 was made after chemical had remained in soil three days, 2 was made after chemical was evaporated, and 3 was cultured after nine weeks' incubation. Nitrates and ammonia were determined after nine weeks' incubation.

[b] Nitrates in milligrams per 100 gm. of soil.

TABLE IV.—*Effect of carbon disulphid and toluol upon Azotobacter and nitrate accumulation in soil*—Continued

WATER CONTENT OF SOIL, 10 PER CENT

Treatment.	Azotobacter film.			NO_3[b].	NH_3.
	1[a]	2[a]	3[a]		
				Mgm.	
Control, 0 cc	Typical	Typical	Typical	15.0	Trace.
Do.	do	do	do	15.5	Do.
Carbon disulphid:					
0.10 cc	do	do	do	15.0	Do.
0.10 cc	do	do	do	15.0	Do.
0.25 cc	do	do	do	16.5	Do.
0.25 cc	do	do	do	17.2	Do.
0.50 cc	do	do	do	15.5	Do.
0.50 cc	do	do	do	17.2	Do.
1.00 cc	do	do	do	16.0	Do.
1.00 cc	do	do	do	16.0	Do.
5.00 cc	None	None	None	6.4	Abundant.
5.00 cc	do	do	do	15.0	Do.
Toluol:					
0.10 cc	Typical	Typical	Typical	16.0	Trace.
0.10 cc	do	do	do	16.0	Do.
0.25 cc	do	do	do	15.0	Do.
0.25 cc	do	do	do	15.0	Do.
0.50 cc	do	do	do	16.5	Do.
0.50 cc	(?)	(?)	(?)	15.5	Do.
1.00 cc	None	None	None	14.4	Do.
1.00 cc	do	do	do	15.0	Do.
5.00 cc	do	do	do	12.0	Abundant.
5.00 cc	do	do	do	9.0	Do.

WATER CONTENT OF SOIL, 20 PER CENT

Control, 0 cc	Typical	Typical	Typical	12.6	Trace.
Do.	do	do	do	12.6	Do.
Carbon disulphid:					
0.10 cc	do	do	do	12.7	Do.
0.10 cc	do	do	do	12.6	Do.
0.25 cc	do	do	do	13.0	Do.
0.25 cc	do	do	do	13.0	Do.
0.50 cc	do	do	do	13.2	Do.
0.50 cc	do	do	do	12.0	Do.
1.00 cc	do	do	do	11.4	Slight.
1.00 cc	do	do	do	12.0	Do.
5.00 cc	None	None	None	5.5	Abundant.
5.00 cc	do	do	do	6.0	Do.
Toluol:					
0.10 cc	Typical	Typical	Typical	12.6	Trace.
0.10 cc	do	do	do	16.0	Do.
0.25 cc	do	do	do	13.0	Do.
0.25 cc	do	do	do	12.2	Do.
0.50 cc	do	do	do	13.0	Do.
0.50 cc	(?)	(?)	do	13.2	Do.
1.00 cc	None	None	None	13.0	Do.
1.00 cc	do	do	do	13.2	Do.
5.00 cc	do	do	do	10.9	Abundant.
5.00 cc	do	do	do	9.6	Do.

[a] 1, 2, and 3 have reference to time at which cultures were made. 1 was made after chemical had remained in soil three days, 2 was made after chemical was evaporated, and 3 was cultured after nine weeks' incubation. Nitrates and ammonia were determined after nine weeks' incubation.

[b] Nitrates in milligrams per 100 gm. of soil.

In another experiment a local soil which had shown vigorous Azotobacter development and nitrogen fixation was used. The moisture content varied in different samples, being 3 per cent, 12 per cent, 24 per cent, and 36 per cent, respectively. After three days' treatment with carbon disulphid and toluol the samples to be evaporated were treated as in the preceding experiment. The moisture content of those samples containing 24 per cent of water decreased very slowly, it being necessary to leave them for two weeks before they were air-dry. The other evaporated samples were left the same length of time before the moisture was made up to the required amount. No attempt was made to evaporate the samples containing 36 per cent of water. After evaporation the moisture content of all samples, except those containing 36 per cent, was made up to 24 per cent. During incubation the loss of water due to evaporation was restored.

Cultures for Azotobacter were made six weeks after the soil had been treated, and seven weeks later the nitrate content was determined. A number of quantitative nitrogen determinations were lost through accident, but the qualitative results are sufficient for present needs. The results are given in Tables V and VI.

TABLE V.—*Effect of carbon disulphid upon nitrogen fixation and nitrate accumulation*

NOT EVAPORATED

Treatment.	Water content, 3 per cent.				Water content, 12 per cent.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b]	NH_3.
		Mgm.	*Mgm.*			*Mgm.*	*Mgm.*	
Control, 0 cc.....	Typical...	9.10	30.8	Trace.....	Typical...	9.05	25.0	Trace.
Do.....	...do.....	10.40	33.3	.....do.....	...do.....	8.75	24.9	Do.
Carbon disulphid;								
0.25 cc.....	.....	.....	.....	.....	...do.....	8.65	23.4	Do.
0.25 cc.....	.....	.....	.....	.....	...do.....	9.35	25.8	Do.
0.50 cc.....	Typical...	10.90	33.3	Trace.....	...do.....	8.75	24.0	Do.
0.50 cc.....	...do.....	9.55	.....	.....	...do.....	8.70	24.9	Do.
1.00 cc.....	...do.....	10.20	35.4	Trace.....	...do.....	9.30	27.2	Do.
1.00 cc.....	...do.....	9.30	33.3	.....do.....	...do.....	9.30	25.8	Do.
5.00 cc.....	...do.....	9.20	35.0	.....do.....	None.....	1.90	27.2	Do.
5.00 cc.....	None.....	4.65	32.1	.....do.....	...do.....	2.15	15.0	Abundant.
10.00 cc.....	...do.....	2.25	23.4	Abundant...	.....	.....	.....	.....
10.00 cc.....	...do.....	1.70	18.6	4.3 Mgm....	.....	.....	.....	.....

EVAPORATED

Control, 0 cc.....	Typical...	10.50	30.0	Trace.....	Typical...	8.65	27.2	Trace.
Do.....	...do.....	9.40	34.8	.....do.....	...do.....	8.75	30.0	Do.
Carbon disulphid:								
0.25 cc.....	.....	.....	.....	.....	...do.....	8.90	27.2	Do.
0.25 cc.....	.....	.....	.....	.....	...do.....	9.25	33.3	Do.
0.50 cc.....	Typical...	9.65	35.4	Trace.....	...do.....	9.90	33.3	Do.
0.50 cc.....	...do.....	9.80	33.3	.....do.....	...do.....	9.45	28.2	Do.
1.00 cc.....	...do.....	9.95	33.3	.....do.....	...do.....	10.25	33.3	Do.
1.00 cc.....	...do.....	9.45	33.3	.....do.....	...do.....	9.75	28.2	Do.
5.00 cc.....	...do.....	9.15	35.4	.....do.....	(?)	6.45	37.5	Do.
5.00 cc.....	...do.....	10.55	33.3	.....do.....	None.....	2.45	17.4	Abundant.
10.00 cc.....	None.....	4.25	.....	.....	.....	.....	.....	.....
10.00 cc.....	...do.....	2.30	30.0	Trace.....	.....	.....	.....	.....

[a] Nitrogen fixed in milligrams per 50 cc. of culture. [b] Nitrates in milligrams per 100 gm. of soil.

TABLE V.—*Effect of carbon disulphid upon nitrogen fixation and nitrate accumulation*—Continued

NOT EVAPORATED

Treatment.	Water content, 24 per cent.				Water content, 36 per cent.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.	Azotobacter film.	Nitrogen fixed.	NO_3.	NH_3.
		Mgm.	*Mgm.*			*Mgm.*	*Mgm.*	
Control, 0 cc	Typical	9.30	37.5	Trace	Typical	9.40	42.8	Trace.
Do	do	9.75	32.1	do	do	9.50	37.5	Do.
Carbon disulphid:								
0.25 cc	do	9.55	37.5	do	do	8.85	37.5	Slight.
0.25 cc	do	9.20	33.3	do	do	8.45	40.8	Trace.
0.50 cc	do	9.50	35.4	do	(?)	3.25	40.0	Do.
0.50 cc	do	8.65	31.0	do	Typical	9.30	40.8	Do.
1.00 cc	do	10.30	33.3	do	(?)	5.95	42.8	Do.
1.00 cc	do	9.65	33.3	do	Typical	9.40	36.0	Do.
5.00 cc	None	3.10	20.0	Abundant	None	2.40	23.4	Abundant.
5.00 cc	do	2.45	18.8	5.0 Mgm.	do	2.35	20.4	4.8 Mgm.
10.00 cc								
10.00 cc								

EVAPORATED

Control, 0 cc	Typical	9.15	37.5	Trace				
Do	do	9.25	36.0	do				
Carbon disulphid:								
0.25 cc	do	9.10	38.8	do				
0.25 cc	do	9.85	32.1	do				
0.50 cc	do	9.90	35.4	do				
0.50 cc	do	8.00	34.8	do				
1.00 cc	do	9.35	36.4	do				
1.00 cc	do	8.40	34.8	do				
5.00 cc	None	1.85						
5.00 cc	do	2.05	19.6	5.4 Mgm.				
10.00 cc								
10.00 cc								

[a] Nitrogen fixed in milligrams per 50 cc. of culture. [b] Nitrates in milligrams per 100 gm. of soil.

TABLE VI.—*Effect of toluol upon nitrogen fixation and nitrate accumulation*

NOT EVAPORATED

Treatment.	Water content, 3 per cent.				Water content, 12 per cent.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.
		Mgm.	*Mgm.*			*Mgm.*	*Mgm.*	
Control, 0 cc	Typical	9.10	30.8	Trace	Typical	9.05	25.0	Trace.
Do	do	9.90	33.3	do	do	8.75	24.9	Do.
Toluol:								
0.25 cc					do		35.0	Do.
0.25 cc					do		28.2	Do.
0.50 cc	Typical	10.00	37.5	Trace	do	9.70	35.4	Do.
0.50 cc	do	10.55	34.8	do	do			
1.00 cc	do		37.5	do	do	10.90	33.3	Trace.
1.00 cc	do		32.1	do	(?)	4.75	33.3	Do.
5.00 cc	None		37.5	Slight	None	3.20	23.4	Abundant.
5.00 cc	do		34.8	0.8 mgm.	do	2.40	20.4	4.6 mgm.
10.00 cc	do	2.65	21.4	Abundant				
10.00 cc	do		18.8	4.8 mgm.				

[a] Nitrogen fixed in milligrams per 50 cc. of culture. [b] Nitrates in milligrams per 100 gm. of soil.

TABLE VI.—*Effect of toluol upon nitrogen fixation and nitrate accumulation*—Contd.

EVAPORATED

Treatment.	Water content, 3 per cent.				Water content, 12 per cent.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.
		Mgm.	*Mgm.*			*Mgm.*	*Mgm.*	
Control, 0 cc	Typical	10.50	30.0	Trace	Typical	8.65	27.2	Trace.
Do	do	9.40	34.8	do	do	8.75	30.0	Do.
Toluol:								
0.25 cc					do	9.80	37.5	Do.
0.25 cc					do	8.70	32.1	Do.
0.50 cc	Typical		33.3	Trace	do	9.60	40.0	Do.
0.50 cc	do		32.1	do	do	8.65	38.4	Do.
1.00 cc	do		33.3	do	None	2.05	37.5	Do.
1.00 cc	do		31.0	do	do	1.80	21.4	Abundant.
5.00 cc	do		27.2	do	do	2.25	21.4	Do.
5.00 cc	do		30.0	do	do	3.25	18.0	5.7 mgm.
10.00 cc	do							
10.00 cc	do		28.6	Trace				

NOT EVAPORATED

Treatment.	Water content, 24 per cent.				Water content, 36 per cent.			
	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.	Azotobacter film.	Nitrogen fixed.[a]	NO_3[b].	NH_3.
		Mgm.	*Mgm.*			*Mgm.*	*Mgm.*	
Control, 0 cc	Typical	9.30	37.5	Trace	Typical	9.40	42.8	Trace.
Do	do	9.75	32.1	do	do	9.50	37.5	Do.
Toluol:								
0.25 cc	do	9.40	37.5	do	None	5.85	45.0	Do.
0.25 cc	do	9.50	34.8	do	do	4.30	45.0	Do.
0.50 cc	do	9.80	37.5	do	do	3.75	46.2	Do.
0.50 cc	do	10.00	34.8	do	do	1.75	47.4	Do.
1.00 cc	None	2.75	38.8	do	do	2.20	30.0	Abundant.
1.00 cc	do	2.60	34.8	do	do	2.00	33.3	3.6 mgm.
5.00 cc	do	2.00	25.0	Abundant	do	1.75	23.4	Abundant.
5.00 cc	do	2.30	22.5	5.7 mgm	do	2.20	22.5	6.6 mgm.
10.00 cc								
10.00 cc								

EVAPORATED

Control, 0 cc	Typical	9.15	37.5	Trace				
Do	do	9.25	36.0	do				
Toluol:								
0.25 cc	do	9.75	37.5	do				
0.25 cc	do	9.35	34.8	do				
0.50 cc	do	9.60	37.5	do				
0.50 cc	do	9.85	37.5	do				
1.00 cc	None	2.80	37.5	Slight				
1.00 cc	do	2.50	34.8	0.9 mgm				
5.00 cc	do	2.20	20.6	Abundant				
5.00 cc	do	2.25	18.8	5.4 mgm				
10.00 cc								
10.00 cc								

[a] Nitrogen fixed in milligrams per 50 cc. of culture. [b] Nitrates in milligrams per 100 gm. of soil.

It will be observed that in the experiments the results of which are presented in Table IV the highest quantity of carbon disulphid and toluol used was without effect upon either Azotobacter or nitrate accum-

ulation in the air-dry soil. For the soil containing 10 per cent of water 5 cc. of carbon disulphid and 1 cc. of toluol were sufficient to destroy Azotobacter, while 5 cc. of either were sufficient to check nitrate accumulation; 0.5 cc. of toluol checked Azotobacter in one sample, but was not sufficient to kill. The results for the soil containing 20 per cent of water are in every respect similar to that containing 10 per cent. In all cases there was an accumulation of ammonia only when nitrification was checked.

The only air-dry samples in which carbon disulphid destroyed Azotobacter were those treated with 10 cc. In one unevaporated sample 5 cc. eliminated Azotobacter, although in the duplicate no elimination occurred. Ten cc. checked the nitrate accumulation in unevaporated samples, but were ineffective where the carbon disulphid was evaporated.

When the water content was 12 per cent, 5 cc. of carbon disulphid were sufficient to destroy Azotobacter and to check nitrate accumulation. This also is true of the soil containing 24 per cent of water. Where the water content was 36 per cent, the results are somewhat irregular. Azotobacter were destroyed and nitrate accumulation was checked when treated with 5 cc.; however, 0.5 and 1.0 cc. appeared to affect the Azotobacter in one instance each, but was without effect upon the nitrate accumulation. This irregularity was probably due to reinoculation in the case of those samples in which duplicates did not agree. The results agree in showing an accumulation of ammonia only in those samples in which nitrification was checked.

The results with toluol as given in Table VI show that in the air-dry soil 5 cc. were sufficient to destroy Azotobacter in the nonevaporated samples, while 10 cc. were insufficient in the evaporated samples. Apparently nitrate accumulation was checked to an appreciable extent with 10 cc. in nonevaporated samples, but was not affected when the toluol was evaporated from the soil. One cc. of toluol was sufficient to destroy Azotobacter in evaporated and in one nonevaporated sample when the water content was 12 per cent, while 5 cc. were required to affectively check nitrate accumulation. In one evaporated sample 1 cc. checked the nitrate accumulation.

When the moisture content was raised to 24 per cent, 1 cc. destroyed Azotobacter in all samples, while 5 cc. were still required to check nitrate accumulation to any appreciable extent.

With a moisture content of 36 per cent, 0.25 cc. of toluol destroyed Azotobacter, and 1 cc. was sufficient to check nitrate accumulation.

As in all other experiments ammonia accumulated only when nitrification had been checked.

It appears from these results that both carbon disulphid and toluol will check nitrate accumulation if applied in sufficient quantities. The quantity necessary to bring about this effect varies quite widely with the physical condition of the soil and probably also with different soils.

The quantity necessary to destroy the nitrifying organisms is very much larger than that which is necessary to check their activilty. There are some times slight increases in the accumulation of nitrate is treated as compared to nontreated soils. This increase, however, can not be attributed to a stimulation of the nitrifying organisms.

There is never an accumulation of ammonia unless nitrification has been checked, which means that in no instance does the process of ammonia formation exceed that of nitrification, the latter process being limited by the former. Any increase in nitrate formation, therefore, merely means an increased ammonia formation. In those samples in which nitrification has been checked and there is an accumulation of ammonia the total nitrogen present as nitrate and ammonia is not materially different from that present in other samples.

The effect of carbon disulphid and toluol upon the Azotobacter group of organisms is more definite and pronounced than it is upon the nitrifying organisms. This is due to the fact that if the application is sufficient to have any appreciable effect, it appears to actually destroy the organisms rather than to check their activity.

Again the effect of both carbon disulphid and toluol appears to depend more upon the condition of the soil than upon the quantity applied. The data show that under some conditions an application of many times as much is necessary to destroy Azotobacter as under other conditions. For these reasons no definite statement can be made as to the effect a given application will have. One-tenth cc. of carbon disulphid per 100 gm. of soil has been observed to destroy Azotobacter completely, while in other instances 5 cc. were without effect. Similarly 0.25 cc. of toluol has been observed to kill, while under other conditions 10 cc. were without effect.

The elimination of Azotobacter does not eliminate nitrogen fixation. There are other nitrogen-fixing organisms that are able to withstand the heaviest application of carbon disulphid and toluol we have made. The quantity of nitrogen fixed in the absence of Azotobacter is usually from one-fourth to one-half that when they are present, and the quantity fixed appears to decrease as the application of carbon disulphid and toluol increases.

As soon as the wide difference in the quantity of carbon disulphid and toluol necessary to destroy Azotobacter under different soil conditions was observed, the question of the cause of such differences very naturally arose. As mentioned in the experimental part of this paper, the moisture content offered a working basis.

In commenting upon the writer's earlier work with carbon disulphid and toluol Kelley [1] suggested that, since these substances are only slightly miscible with water, the noneffectiveness of these agents in decreasing the number of bacteria was possibly due to the high-moisture

[1] KELLEY, W. P. AMMONIFICATION AND NITRIFICATION IN HAWAIIAN SOILS. Hawaii Agr. Exp Sta Bul. 37, 52 p. 1915.

content used in our experiments. The high-moisture content, according to Kelley, would prevent carbon disulphid and toluol from coming in contact with the organisms. The results herein reported appear to show that this is not true.

A study of the literature devoted to the question of partial sterilization by means of volatile antiseptics reveals a mass of conflicting data and numerous contradictions and criticisms. As far as ascertained, the influence of moisture upon such treatment has been considered by only one investigator. Greig-Smith [1] found that the effct of toluol upon soil protozoa varied with moisture content. The available review of this paper gives no data except the statement that 20 per cent of toluol failed to destroy certain protozoa if the moisture content were less than one-tenth saturation, but if the moisture content was above this 1 or 2 per cent of toluol was sufficient to kill them. In the soils used any degree of saturation below one-tenth would approach very closely an air-dry condition. It appears, then, that the experiments reported in this paper agree in general with Greig-Smith's. It is possible that many of the conflicting results heretofore reported as to the effect of volatile antiseptics, or so-called partial sterilization, may be explained solely upon the moisture content of soil when such treatment was made.

SUMMARY

Carbon disulphid and toluol when applied to soils in sufficient quantities will destroy Azotobacter group of organisms and check the accumulation of nitrate nitrogen and possibly will destroy the nitrifying organisms.

The quantities necessary to produce such effects vary quite widely depending among other things upon the quantity of moisture present.

Apparently if the quantity of carbon disulphid or toluol is sufficient to have any effect upon Azotobacter they are usually completely destroyed. On the other hand, there is a great difference in the quantity necessary to destroy nitrifying organisms and that necessary to check their activity.

There are nitrogen-fixing organisms other than Azotobacter present in soils which are not destroyed with 10 cc. of carbon disulphid or toluol even when the moisture content of the soil is high.

Following treatment with carbon disulphid and toluol there is no appreciable accumulation of ammonia unless nitrification has been checked.

There is no evidence in these experiments to show that treatment with antiseptics stimulates the nitrifying organisms, and there is little evidence to indicate a stimulative effect upon the ammonifying or nitrogen-fixing organisms.

[1] GREIG-SMITH, R. THE ACTION OF TOLUENE UPON SOIL PROTOZOA. (Abstract.) In Nature [London], v. 94, no. 2360, p. 581. 1915.

A MULTIPLE-PIPETTE HOLDER FOR THE DISTRIBUTION OF SERUM FOR THE COMPLEMENT-FIXATION TEST

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DISADVANTAGES OF FORMER METHOD

Those engaged in the performance of complement-fixation tests, especially in laboratories where such work is conducted on a large scale, readily can appreciate the necessity for the so-called "short-cuts" consistent, of course, with accuracy, and one's imagination can readily appreciate the tediousness of removing the serums for diagnosis from about 1,000 (daily average) and at times as many as 2,400 specimens. The transferring of the serums from the bottles to the test tubes has been long a matter of concern and required the employment of many assistants, and even then it would consume the greater part, if not all, of the forenoon in the distribution of the large number of samples.

It might be stated here that the desired quantities of serum are placed in test tubes containing 1.5 cc of physiological salt solution and inactivated for half an hour at 52° C, the other ingredients being added thereto later.

The pipettes employed in the measuring of the necessary amounts of serum were those of the 1-cc. variety, made of glass, and graduated into tenths and hundredths. Such pipettes are filled by suction with the mouth and controlled by the index finger, and although one may become quite expert in the handling of the same, the constant reading of the smaller graduations is not conducive to good vision. Such pipettes are not infrequently of a larger diameter than the opening in the serum bottle, their use in such cases requiring the uptilting of the bottle with consequent agitation of its contents, a feature not at all desirable; and, further, the utilization of this method of serum measurement meant the consuming of several hours and the assistance of numerous operators.

Necessity therefore prompted the creation of a device which has overcome all the objectionable features above enumerated and has made possible the transferring of serums from bottles to test tubes a matter of comparative ease, requiring but a short space of time and demanding but few operators, all of which are especially desirable in this era of time and labor saving.

After deliberation it was concluded that the new device now utilized and illustrated herein would satisfactorily meet the existing conditions

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and was therefore adopted. It is based on the principle of the multiple pipette devised by Buck [1] which is used in this laboratory for the distribution of salt solution, complement, etc.

DETAILS OF NEW DEVICE

The device (fig. 1) consists of a brass tube, 15¼ inches in length and ⅜ inch in diameter (outside), which is tapped at both ends to permit cleaning should occasion arise. The lumen communicates, in its length, with 12 brass collaterals set at equal distances apart and at right angles to the main tube. Each collateral is 2⅜ inches in length with a ¼-inch lumen, and is lined with rubber tubing of sufficient length to project about ½ inch. This free end of rubber tubing is everted over the opening of the metal collateral, thus causing a slight constriction of the lumen of the rubber at that point and serving to fit snugly about the glass pipettes to be fitted therein. On the opposite side of the main tube, at the center and at right angles, is located a single short metal collateral, or mouth piece, to which is attached a piece of rubber tubing for the control of the device and to which a pinch clamp may be applied, although controlling by pressure with the forefinger and thumb has been found to be more convenient.

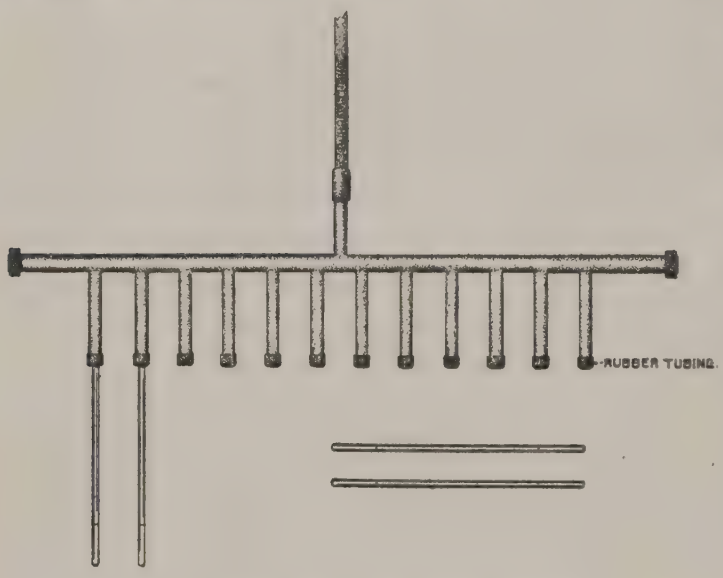

FIG. 1.—General view of device and pipettes.

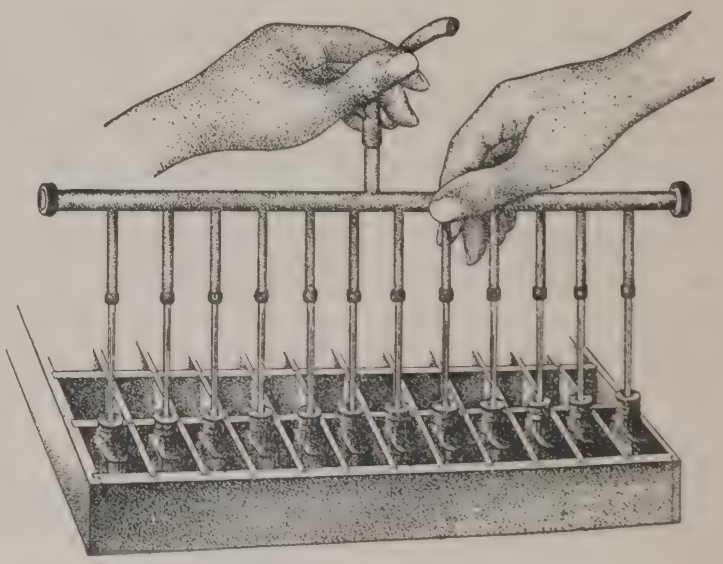

FIG. 2.—Holder connected with serum bottles in standard tray.

[1] BUCK, JOHN M. A MULTIPLE PIPET FOR THE COMPLEMENT-FIXATION TEST. *In* Jour. Infect. Diseases, v. 19, no. 2, p. 267–271, 3 fig., 1916.

The pipettes are made of selected 4-mm. glass tubing and graduated for the proper amount of serum for use in the test. These graduations for convenience are etched at both ends.

The bottles in which the serums are received, although varying slightly in size and shape, have permitted the making of standard trays (fig. 2), so constructed as to hold 144 bottles, 12 bottles wide and deep, and so placed as to register with the pipettes when they are brought into apposition.

Each tray is numbered on the end to indicate its position in the test, while the horizontal rows of bottles are given subnumbers to correspond with their respective test-tube racks. The test tubes are numbered to correspond with the bottles.

The test-tube racks (fig. 3) are constructed to accommodate a double row of 12 tubes each and which, in turn, register with the pipettes. In short, the arrangement of the bottle trays, test-tube racks, and pipettes is standardized.

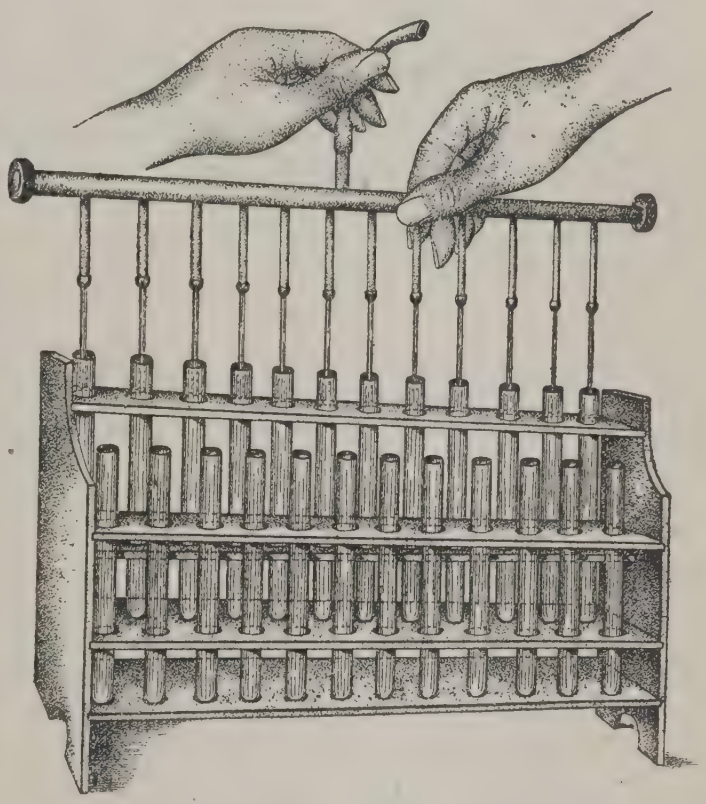

FIG. 3.—Test tubes in rack.

When the stoppers of the serum bottles have been removed and the trays arranged in proper order to correspond with the racks, the operator is handed one of the metal pipette holders containing 12 clean, sterilized pipettes. These are inserted into the 12 bottles of the first row and immersed to just below the surface of the smaller amount when suction is applied to the mouthpiece, and serum from 12 test animals drawn up simultaneously by one operation. The serums are drawn above the etched graduations, then allowed to recede until the graduations are reached. This is done to equalize the amount in each pipette, for it has been found that some bottles contain more than others and when the serums are drawn up the corresponding columns will be higher, but by letting the serum partially escape down to the graduations, the quantities in the pipettes are readily equalized. The serums are then transferred to the 12 test tubes bearing numbers corresponding with those on the bottles.

The holder is then passed to an assistant who removes the used pipettes which are placed in water to prevent drying of the serum, after which fresh pipettes are inserted and equalized by slight pressure on a flat level surface. The use of four metal holders insures always a clean set of pipettes ready for use by the operator.

On completion of the removal of serums, the pipettes are subjected to a thorough washing, drying, and sterilization in hot air for two hours.

Actual tests showed that 1,000 specimens can be removed conveniently from the bottles and placed in test tubes in about one-half hour.

The following advantages are claimed for the holder:

(1) No eyestrain attends the operation of the device.

(2) The pipettes are sufficiently small to enter the bottles without agitation of the contents.

(3) Only one operator and two assistants are required, where previously many were necessary.

(4) Only a short time is needed for one operator to remove specimens of a great number of serum samples.

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PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA

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INTRODUCTION

During the last few years *Cronartium ribicola* Fischer has become the most serious fungus pest of white pine (*Pinus strobus* L.) in America. As such it has been the subject of much study by pathologists who realized, from a knowledge of the course of the parasite in Europe, the damage it was capable of causing. Spaulding (*54*)[1] in the most comprehensive paper on the fungus which has appeared in this country, reviews the reports of its ravages on white pine in the Old World and gives its general characters and life history. Since the discovery by Stewart (*57*) of *C. ribicola* on species of Ribes at Geneva, N. Y., in 1906, many papers have appeared calling attention to the absolute necessity of controlling the spread of this parasite if the white pine is to be saved for reforestation. A great deal of attention has been given to testing the susceptibility of possible hosts through inoculation and to control and eradication methods, both in the United States and Canada. In Europe most of the recent work has been along this same line. Klebahn (*23*, *24*, *25*, *26*) and Tubeuf (*58*, *59*, *60*) have carried on extensive experiments.

The morphology of *Cronartium ribicola* and the details of the interrelations of the parasite and its hosts have never been thoroughly worked out. The cytology of the genus Cronartium has up to the present time received very little attention. In the following paper the results of certain observations extending over a period of two years will be presented, first, with reference to the minute histology of the fungus and the interrelations of host and parasite, and second, with reference to the cytological phenomena accompanying spore production in the different types of sori. The paper is offered as a contribution to our knowledge of the parasitism, morphology, and cytology of the genus Cronartium.

[1] Reference is made by number (italic) to "Literature cited," p. 655–659.

LIFE HISTORY AND HOSTS

The life history of *Cronartium ribicola* has been so well reported by Tubeuf (*59*, *60*), Klebahn (*25*), Spaulding (*54*, *55*), and others that it is only necessary to repeat it in outline by way of introduction to what follows. Sporidia from the teliospores produced on *Ribes* spp. infect young stems and branches of *Pinus strobus* and other 5-needled pines. The pycnial and æcial stages subsequently develop on the pines. Æciospores from the pine infect leaves of *Ribes* spp., on which the uredinia are shortly formed. These sori are produced in successive generations throughout the summer. Telia develop from old uredinia, or as separate entities, in the form of compact columns. The teliospores germinate *in situ*, each one producing a promycelium which gives rise to four sporida. A diagram of the life cycle is presented in text figure 1.[1]

In the United States and Canada the pine most frequently attacked is *Pinus strobus*, although *P. flexilis* James and *P. parviflora* Sieb. and Zucc. have been found infected. Practically every known species of Ribes is susceptible to infection to a greater or less degree, and therefore the discovery of an immune variety is much to be desired. The results of inoculations on *Ribes* spp. in America have been reported by Spaulding and Gravatt (*56*), and further work is being conducted with all the species and varieties of Ribes obtainable.

EXPERIMENTAL METHODS

The method used for rapid examination of specimens of pine suspected of being infected has been described in detail in a previous paper (*7*). This procedure in brief is as follows: Sections from fresh pine bark are cut on an ether freezing microtome, rinsed in water, stained in safranin and *Lichtgrün*, cleared in clove oil followed by xylol, and mounted in balsam. They are then examined, preferably with an oil-immersion lens, to determine the presence or absence of the characteristic mycelium and the striking haustoria of the rust, the latter being especially important from a diagnostic standpoint. This method yields transparent sections which for general morphological study have not been surpassed by following any of the more complicated methods given below. Very little shrinkage occurs in the mycelium, and as the method is usually employed before pycnia and æcia appear, the preservation of the hyphæ in as near their natural shape as possible is practically all that is necessary. Moreover, the host tissue shrinks so slightly that the distortion is entirely negligible.

Two killing agents were used in the preparation of material for paraffin or celloidin embedding: (1) formalin-alcohol, made by adding 6 cc. of full strength commercial formalin (U. S. P. VIII) to 94 cc. of 70 per cent

[1] The drawings for the text figures were made by the aid of a camera lucida and a special projection apparatus; for the drawings on the plates a camera lucida was used. The photographs and photomicrographs are all original. The author is indebted to Miss Minnie W. Taylor, Assistant in Forest Pathology, for the preparation of the drawing for text figure C of plate 50.

alcohol; and (2) Flemming's fluid, both weak and strong. The first was used for pine material only; the second for both pine and *Ribes* spp. Formalin-alcohol gave excellent result not only for gross mycelial characters but also in some cases for nuclear phenomena. The material sec-

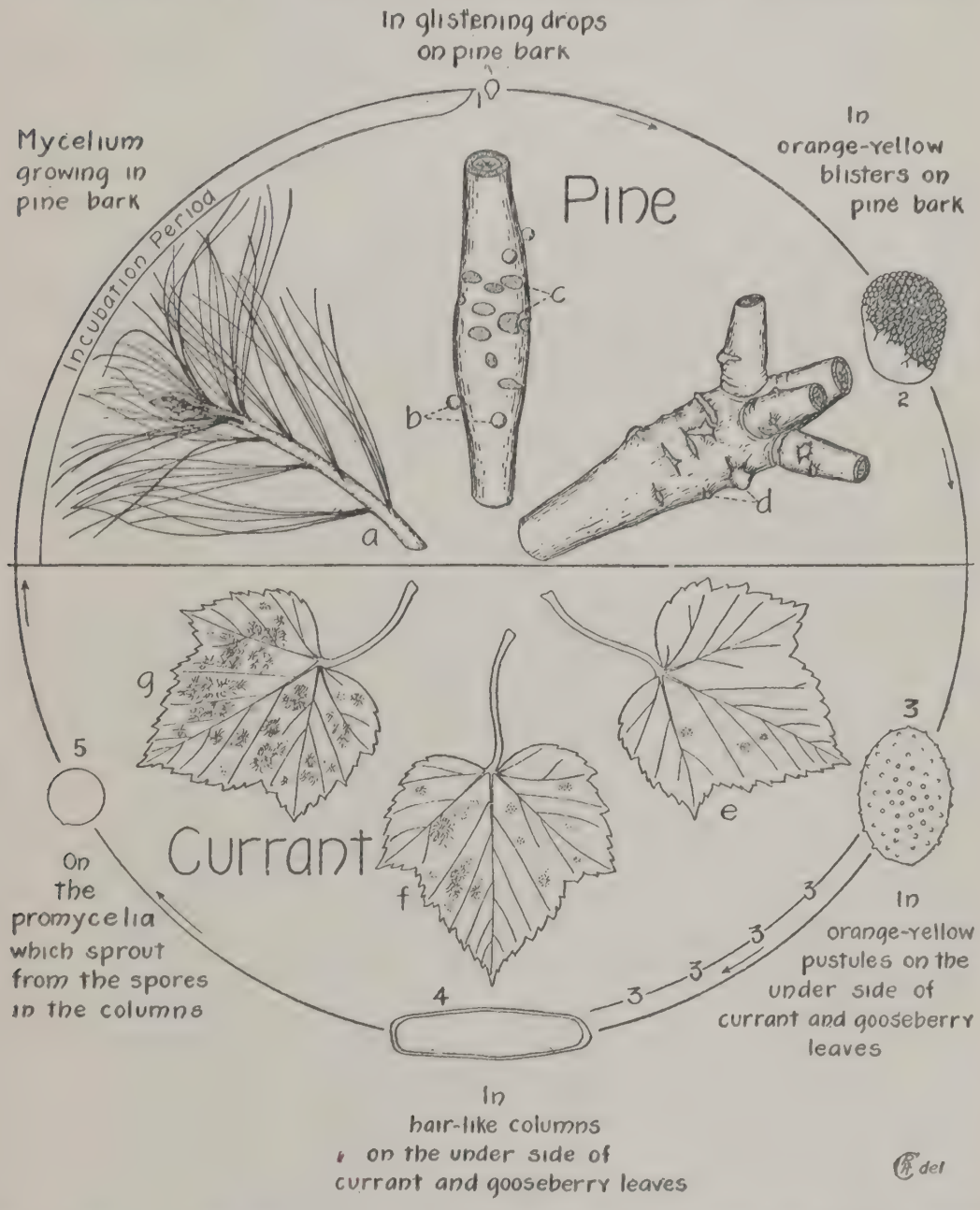

FIG. 1.—Diagram representing the life cycle of *Cronartium ribicola*. The spores are drawn to approximately the same scale and are numbered in the order of their appearance: 1, pycniospore; 2, æciospore; 3, urediniospore; 4, teliospore; 5, sporidium. The repetition of the figure 3 in the broken line between the urediniospore and the teliospore indicates that the urediniospores appear in successive generations in a single growing season. *a*, A young pine branch at the time infection usually takes place: *b*, pycnial drops; *c*, pycnial spots: *d*, æcia; *e*, the dots on the currant leaf represent uredinia; *f*, the dots represent uredinia and the dashes telial columns; *g*, this figure represents the condition of the infected leaf in the fall when the telial columns are the predominant spore forms.

tioned easily, either in paraffin or celloidin, and stained well with safranin and *Lichtgrün* or Haidenhain's iron-alum hematoxylin. Both concentrations of Flemming's fluid were very satisfactory. The material was cut into pieces not over 3 mm. square, fixed for 24 to 48 hours and then

thoroughly washed in running water. Certain generally accepted principles of killing and fixing were considerably modified by greatly shortening the time the material was left in the different reagents. The time the material remained in the grade alcohols was cut to 5 minutes for 10 per cent alcohol, 10 minutes for 15 per cent, 20 minutes for 20 per cent, and 30 minutes each for 30, 40, 50, 70, 80, 90, and 95 per cent. Absolute alcohol was used for 30 minutes with one change.

Xylol-alcohol was used both in short steps of 5, 10, 15, 20, 25, 50, and 75 per cent, or in a 25, 50, and 75 per cent concentration. For the short jumps the schedule of 5 minutes for 5 per cent, 10 minutes for 10 per cent, etc., was adopted; for all the longer jumps 30 minutes were allowed. The longer jumps apparently did not injure the material or its staining qualities. Pure xylol was used for one hour, with one change. The infiltration with paraffin was carried out as rapidly as was feasible, starting with shavings of 45° paraffin in xylol in cold solution, for 12 to 15 hours (overnight), then at the water-bath temperature, gradually adding more soft paraffin, for 24 hours. The mixture of xylol and paraffin was replaced by pure 45° paraffin for two hours, the latter by 55° paraffin for four hours, with one change and the material then embedded in the harder paraffin.

Æciospores and telial columns with promycelia and sporidia attached were killed in Flemming's fluid and handled with the aid of a centrifuge. By using a short spinning at moderate speed to send the small objects to the bottom of the centrifuge tube little material was lost in decanting or pipetting off the different reagents. When the material had been brought to pure paraffin, it was transferred to a glass tube 2 inches long with ¼-inch bore, which had been previously well coated inside with glycerin and stoppered at one end with a close-fitting cork. This piece of tubing was then placed in the centrifuge while hot and the centrifuge started rather rapidly. After about a minute of rapid spinning the centrifuge was slowed down and kept going at a moderate rate until the paraffin was completely cooled. Experience has shown that if the small objects are simply allowed to settle and the tube then cooled in water, the paraffin will solidify first next to the glass and remain fluid in the center with the result that the core of the plug will be hollow when the final contraction and cooling has taken place. The hollow may extend quite down to the cork, making it impossible to cut a clean, well-shaped block for sectioning. The use of the centrifuge completely overcomes this trouble and also crowds the minute objects closer to the bottom of the tube against the cork. When the paraffin has cooled, the cork is pulled out and the paraffin plug pushed out of the tube.

The paraffin method was used exclusively for embedding bark and leaf tissues. It was found that the bark tissue could be easily cut down to 5 μ without resorting to hydrofluoric-acid treatment. Wood was treated

with 50 per cent hydrofluoric acid and then embedded in celloidin,[1] or was simply placed in 95 per cent alcohol and glycerin, equal parts, for about 10 days to 2 weeks, and sectioned without embedding at all. Neither of these two methods gave better sections than could be obtained from fresh tissue with the aid of the ether freezing microtome and a sharp knife, except in the case of tangential sections. A thickness of 10 to 20 μ was found the most favorable for the study of the mycelium in the different elements of the cortex, phloem, and xylem. In sections thinner than 10 μ the mycelial strands were often torn out of place. For the study of the pycnia and æcia sections were used as thin as 3 μ, but 5 to 7.5 μ were generally employed. Sections of uredinia and telia were cut 3, 5, and 7 μ thick.

For mounting, Land's fixative,[2] potassium-bichromate + gum-arabic, was found superior to egg albumen and was used almost entirely for bark sections. It was also found more satisfactory for long sections of the leaf.

A rather long series of stains was employed, and a comparison of the different features of the mycelium and host cells made from slides colored with the different stains. The diagnostic method has already been mentioned. Alcoholic safranin and clove-oil + gentian-violet were particularly good for mycelium in the xylem. Safranin and Delafield's hematoxylin was also a favorable combination for such infected tissue. In the phloem and cortex the mycelium was well differentiated with Delafield's hematoxylin followed by erythrosin in 70 per cent alcohol. For cytological study Haidenhain's iron-alum hematoxylin in combination with aqueous Congo red, aqueous orange G, or *Lichtgrün* in 95 per cent alcohol proved excellent. Host and parasite cell walls stained well with the *Lichtgrün*, whether in combination with the hematoxylin or safranin. Flemming's triple stain [3] gave the best results for cytological study, with one or two exceptions to be mentioned later; and by using a strong violet stain the mycelial and host relations at the bases of the sori were brought out more clearly than with any other combination. The gentian-violet was made up in small quantities, enough for one week, as it does not keep well. All reagents from the safranin to the xylol were handled with pipettes from dropper bottles and used but once.

It was found that material once correctly embedded in paraffin, sectioned, and mounted, would stand the jump from 95 per cent alcohol to water, then a 5-minute immersion in full strength hydrogen peroxid for bleaching, without shrinkage or distortion. The sections were rinsed in

[1] See PLOWMAN, Amon B. THE CELLOIDIN METHOD WITH HARD TISSUES. *In* Bot. Gaz., v. 37, no. 6, p. 456–461. 1904.

[2] See CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. ed. 3, 314 p., illus. Chicago, 1915.

[3] The stains were made up according to the following directions: SAFRANIN, Solution I: 2 gm. of safranin in 95 cc. of 95 per cent alcohol; filter. Solution II: 5 cc. of anilin oil in 95 cc. of distilled water. Shake the two solutions together and filter. GENTIAN-VIOLET, Solution I: 1 gm. of gentian-violet in 15 cc. 95 per cent alcohol. Solution II: 3 cc. of anilin oil in 80 cc. of distilled water. Mix the two solutions, shake, and filter. ORANGE G: 1 gm. of orange G. in 100 cc. of distilled water.

water after bleaching and kept in 70 per cent alcohol until they could be stained. They were stained in safranin for one to two minutes, rinsed in water, differentiated to the proper point with 50 per cent acid alcohol and washed with fresh 50 per cent alcohol. They were then stained for 15 to 60 seconds in gentian-violet. The violet was followed at once by the orange G with the slide held at an angle so that the orange G would run off rapidly and carry the violet with it. Then a few drops of absolute alcohol were mixed with the orange G which remained on the slide to hasten the removal of excess violet, and the mixture was quickly poured off. The mixing process and the pouring off of the mixture were carried out as rapidly as it was possible to manipulate the reagents and the slide. Then a few drops of fresh absolute alcohol were flowed quickly over the slide. The absolute alcohol was replaced by clove oil, the clove oil by xylol, and the mount then finished in balsam. The whole staining and finishing process consumed only a few minutes and the results were far superior to any obtained by following schedules calling for longer staining. The success of the method depends on having good material properly fixed, embedded, and sectioned, and on the use of fresh reagents. The saving in time and the excellence of the finished slides more than make up for the possible excess use of the more expensive alcohols and oils.

The method of using the iron-alum hematoxylin was also shortened by cutting the time in the mordant by from 10 to 30 minutes, and the time in the stain by from 5 to 30 minutes. The results were particularly good for the study of the centrosomes and dividing nuclei. It is obvious that the shorter methods outlined above have distinct advantages over the longer methods generally advocated, provided they yield satisfactory finished products. The short method [1] for the use of Flemming's triple stain is recommended to investigators who have had trouble with this more or less capricious combination.

MORPHOLOGY

INFECTION OF PINE AND EXTERIOR INDICATIONS OF PRESENCE OF PARASITE PREVIOUS TO SPORE FORMATION

The actual process of the entrance of the sporidial germ tube in infecting the pine has not been observed, and indeed the determination of the actual point of natural infection is an exceedingly difficult problem. Practically all infections first become evident by the etiolation or swelling, or both, of the bark at the node or at some point in the internode. The etiolation may be quite marked, as shown in Plate 48, A, representing a recent nodal infection. Internodal infections most often appear to originate at the base of a leaf fascicle. A striking case of this type of

[1] This short method, varied slightly according to the material to be stained, has been very successfully used at the University of Wisconsin and Columbia University, and the author makes no claim for its originality.

infection is shown in Plate 48, B, *a*. McCubbin (*29*) reports that out of 1,007 pine infections examined 925 originated at the bases of leaf fascicles, 14 originated in wounds, and 68 were indeterminate. These conclusions controvert the scarcely tenable theory of Marchal (*35*) that infection generally takes place through wounds. Observations on a large number of infections at Kittery Point, Maine, indicate that most infections can be classed as either nodal or internodal. However, it should be borne in mind that infection probably takes place very often in the bud while the nodal and internodal cells are scarcely differentiated. Therefore the point at which infection appears to originate after the tissues in the bud have elongated may be either nodal or internodal, depending entirely on the chance growth of the sporidial germ tube. In discussing *Peridermium pini* Pers., parasitic on *Pinus sylvestris* L., Hartig (*19*) states that infections with this rust also originate either at the node or the base of the leaf fascicle.

The swelling and etiolation of the bark noted above are the two most prominent indications in early stages after infection of the presence of the parasite. The swelling is confined to the bark alone, the wood actually becoming constricted in old infections, and is due to the fact that the mycelium of the parasite forces apart the phloem and cortex cells of the host. No evidence of any increased division has been observed in the bark cells, and there seems to be no stimulation toward gall formation, such as occurs in other cases—for example, on *Pinus virginiana* Mill. under the attack of *Peridermium cerebrum* Peck. In the case of the nodal infection in Plate 48, A, it will be noted that there is little or no swelling; but, as stated above, both swelling and etiolation may occur at the same time. The irregular edge of the etiolated area marks fairly definitely the advancing tips of the invading hyphæ, which generally extend a little beyond the line.

Other less common external indications of the parasite are bunched needles on seedlings or transplants and occasionally on older trees, and short adventitious branches which spring from the infected nodes. It frequently happens that certain secondary fungus parasites become established on the area already attacked by the rust, and suppress the latter so completely that spore production is partly or completely inhibited. Under these conditions the bark dies and shrinks more rapidly than under the attack of the rust alone, and the stem then appears to be constricted, more or less irregularly, at the infected part. All of these external indications are valuable aids to early recognition of *Cronartium ribicola*.

The period of incubation, from the time of infection to spore production, varies from one to several years, depending possibly on whether the infection takes place in early summer, and therefore under favorable conditions for growth before winter, or in early fall, when growth has unquestionably slowed down. The growth of the mycelium in the bark

in some cases seems to vary directly as the succulence of the host. It is at this time impossible to determine what part weather conditions play, although it seems evident that, once infection has taken place, external conditions cause little or no change in the growth of mycelium. The time of production of æcia in the spring varies directly with warm and favorable weather conditions.

MYCELIUM IN PINUS STROBUS

A brief summary of the main characteristics of the mycelium and the relation of the parasite to the cells of the cortex and phloem of the pine host has already been published (7); however, both subjects will bear review in greater detail. No data can be given at this time on the manner in which the mycelium enters the host tissue, because the youngest stages of infection have not been observed. The course of the hyphæ is always intercellular (Pl. 53). Conditions found at the edge of the infected area indicate that the fungus makes its advance first in the most recent phloem parenchyma and in the rays. This holds true for infections of several years' standing, as well as for those which, to judge from the size of the diseased area, could not have been more than a year old. The hyphæ are relatively large, 3 to 5.5 μ in diameter, and are divided by cross walls into cells which vary considerably in length (Pl. 53). Each cell has a single conspicuous nucleus. In transverse and radial sections the hyphæ can be traced along the rays past the cambium into the xylem (Pl. 53). Here they are confined to the region of the ray cells, with the exceptions noted below. Because of difficulties in cutting transverse sections, which are at right angles not only to the "grain" of the phloem but also to the general course of the hyphæ, such sections are not favorable for study, except in comparison with other sections cut tangentially or radially. In tangential longitudinal sections of the cortex and phloem the mycelium is frequently found in strands, which are especially prominent in the outer phloem region. The strands may surround groups of the phloem parenchyma. In some cases the older sieve cells appear to be filled with hyphæ; but careful observation shows that they have been split apart and flattened out by the hyphæ, which have forced their way into the enlarged intercellular space, and thus practically occupied the same amount of space that was formerly occupied by the living sieve cells. In the rays the hyphæ frequently fill the intercellular spaces adjacent to the horizontal walls of the ray cells, and haustoria from these hyphæ penetrate the ray cells. Resin cells are also penetrated by haustoria. In the xylem the same general conditions are met with in respect to the ray cells that are found in the phloem. Short haustoria arising from the hyphæ in the angles between these ray cells bore through the thin walls of the adjacent tracheids and enter the lumens

of the tracheids [1] for a short distance (Pl. 49, C). Such haustoria are naturally limited in development.

Radial longitudinal sections are more easily cut from bark or wood than transverse or tangential longitudinal sections because radial cuts are splitting cuts. Moreover, in radial sections the vertical widths of the rays are exposed, and therefore all the infected cells of the rays, with the haustoria they contain, as well as the adjacent infected phloem and xylem cells, are more readily examined (Pl. 53). The hyphæ lying along one phloem ray frequently are united with similar hyphæ lying along adjacent rays by connecting strands. The latter may work their way between phloem parenchyma or sieve tubes. In the xylem the ray hyphæ may be connected in the same way by hyphæ, which pass from one ray to the other in the intercellular spaces between the tracheids. Often the edge of the ray is bordered by a hypha lying in the space between the outer ends of the marginal cells of the ray and the tracheids (Pl. 53). In the resin-duct parenchyma and the cells lining the duct all the cells are usually penetrated by haustoria (Pl. 53). This applies to vertical ducts in the wood and bark and to horizontal ducts in fusion with rays.

The general features of the mycelium such as their more or less uniform diameter, with occasional bulges where there is a little extra room in an intercellular space, their relatively large size, uninucleate cells, and constant relations to the host cells of the different tissues, are characteristic enough to make the mycelium alone a sufficient basis for the recognition of *Cronartium ribicola* before any spores are produced. The haustoria often the most striking objects in the infected cells, are the most important elements of the mycelium from a diagnostic standpoint. The haustoria apparently have the power to pierce the cell wall at any point (Pl. 53). Young haustoria are usually straight (Pl. 58, B), constricted at the point of passage through the wall, and irregularly bulging inside the cell (Pl. 53; 58, C, D). Their outline soon becomes more or less irregular. In the phloem parenchyma they do not reach the development found in the ray cells, where they coil on a wide spiral or curve at rather sharp angles. The curve and the spiral are probably different expressions of the same process—that is, the adjustment between the growing haustorium and the cytoplasm of the host cell. As Sappin-Trouffy (*50*, *51*) has pointed out, the haustoria of the rusts appear to seek out the nucleus of the host cell, and sometimes even entwine it. No such extended development has been observed in the case of the haustoria under discussion, but it frequently happens that the host nucleus is dented (Pl. 53) by the tip of the haustorium. Olive (*42*) shows that the haustoria of *Botryorhiza hippocrateae* Whetzel and Olive form botryose masses which may almost completely fill the host cell. In this fungus the haustoria

[1] In a previous article (*7*) the statement was made that haustoria do not enter the wood cells. Improved technic has shown this statement to be an error.

are multinucleate; in *C. ribicola* they are apparently always uninucleate (Pl. 53). In the latter fungus no case of more than one cell in a haustorium has been observed by the writer, although as many as three tips are not uncommonly seen. It is difficult to determine whether the haustorium is always cut off from the hypha from which it is originated and this point will have to be left undecided.

When properly stained with any of the combinations given on page 623, a sheath can be made out at the base of each haustorium (Pl. 58, C, D), enveloping it for a distance of several microns, as if the haustorium were set in a cuplike holder. The sheath really extends all over the haustorium, but is generally very thin in the middle region. At the tip it is as thick or thicker than at the base (Pl. 58, E). In its staining reaction the sheath resembles the wall of the host cell. There is at least a possibility that it is formed by the host cytoplasm in response to the irritation or stimulus caused by the presence of the haustorium. Olive (*42*) states that the plasma membrane of the host protoplasm is pushed in by the haustorium of *Botryorhiza hippocrateae* as it invades the host cell. Apparently the cytoplasm shrinks away from the advancing haustorium in some cases, leaving an appreciable space. This phenomenon has not been observed in *Cronartium ribicola*, but it is certain that the plasma membrane of the host cell is not broken nor pierced by the haustorium; It must be pushed in as the tip of the haustorium grows. The greatest points of irritation produced by the haustorium in the host cell would be the point in the cell wall through which the haustorium entered and the point of contact of the advancing haustorial tip with the host cytoplasm. Consequently it might be assumed that the greatest results from the irritation would be observed at these two points—namely, at the base of the haustorium and at its tip. At these two points the sheath is thicker than at others. Possibly the narrow space which must occur between the haustorium and the host cytoplasm membrane is the dumping ground for precipitation products resulting from the irritation. These products might constitute the beginning of the sheath which would gradually increase in thickness as the age of the haustorium increased.

The sheath certainly does not come into being at the time the haustorium penetrates the wall, as Smith (*53*) has reported for the haustorial sheaths of the Erysipheae. It has not been found in connection with young haustoria in any case and seems to be an accompaniment of maturity or old age. The hole in the host cell wall, through which the young haustorium passes, is comparatively small (Pl. 58, C, D) and at first the wall is no thicker at this point than at any other. Whatever thickening takes place at the point of penetration occurs after the haustorium has entered the cell.

The young haustorium is full of cytoplasm, with a typical round nucleus (Pl. 58, B). As the wall of the haustorium, which is at first

quite thin, thickens and the sheath develops, the cytoplasm becomes vacuolate (Pl. 58, C, D, E), and the nucleus loses its normal structure, condensing into a shrunken deeply staining mass. In such a state the haustorium can not function efficiently as an absorbent organ. At any rate the host cell and its nucleus do not show much evidence that the parasite seriously interferes with the normal cell growth. Even when the cells are completely separated by mycelial stands, the host nuclei may remain apparently normal in shape and structure.

PYCNIA

On any given area of infection the pycnia precede the æcia, by at least one growing season. Succeeding generations of pycnia and æcia follow a more or less definite schedule. The plan of the advance of the fungus is illustrated in Plate 54, A. Immediately surrounding the point of infection the bark may show pycnia and æcia at the same time (Pl. 48, B), but in such cases the pycnia have either passed maturity or the æcia have developed abnormally early. After the infection has spread for some distance, provided that the tree is not less than several inches in diameter, the pycnial area is normally always in advance of the æcial area. The sequence of the etiolated bark, pycnial area, and æcial area is evident in Plate 54, A.

Very young pycnia are difficult to find. They seem to develop rapidly when once started, for none have been examined which were mature in the center and still young at the periphery, a condition which is commonly met in æcia and uredinia. The hyphæ which contribute to formation of the pycnium force their way between the cells of the outer cortex in a direction at right angles to the outer surface of the bark (Pl. 49, B, *a*). They are aggregated between the outermost cortex cells and the periderm, forming a layer of pseudoparenchyma two to four cells thick (Pl. 49, B, *b*). From this layer arises a series of short branching trunks (Pl. 58, A, *c*). Each of the ultimate branches from these trunks is a long sporophore, on the tip of which a number of pycniospores are formed, one after another (Pl. 49, A, B; 58, A, *a*, *b*). Roughly speaking, the pseudoparenchyma makes up one-fourth, the short branching trunks one-fourth, and the sporophores one-half of the vertical width of the sorus (Pl. 49, B, 58, A). Pycniospores are produced in large numbers. Mixed with a thin sweet gelatinous fluid, they collect between the sporophore tips and the periderm layer (Pl. 49, A) forcing the latter up into the form of a shallow blister. Finally a small break in the periderm layer allows the spores to escape, together with the sweet fluid, in the form of a honey-colored drop called the pycnial drop (Pl. 48). The pycniospores are typically pyriform (Pl. 58, A, *b*), measuring when mature approximately 2.5 by 3.5 μ. Their mode of formation appears to agree closely with that of the pycniospores in other rusts. At the rounded tip of the sporophore a bud is formed which swells until it reaches the size of a mature spore

(Pl. 58, A). After receiving its nucleus the spore is abstricted. No evidence of a collar like that figured by Blackman (*2*) was seen, unless the odd constriction of the cytoplasm shown in Plate 58, A, *c*, can be considered as a collar. This phenomenon is quite common. Occasionally long hyphal filaments grow some distance out beyond the tips of the sporophores. The structure of the spores is typical of pycniospores in general. They appear to be completely nonfunctional. No attempt was made to germinate them.

The dark areas on the bark which indicate the location of the pycnia are designated pycnial spots (Pl. 54, A, *b;* 48, B). They are honey-yellow to brown-yellow at first, but they gradually assume a color like that of clotted blood as the pycniospores mature and ooze out, and may finally become almost black. Later, when the covering of cork cells sloughs off or is eaten off by insects, the drying cells of the host tissue beneath turn a typical light pink color. After reaching maturity the pycnium is cut out from the host tissue by the formation of a cork cambium and the deposition of a cork layer at a depth of four to six cells below the bottom of the pseudoparenchyma base of the sorus (Pl. 50, C, *b*). It is the exposure of this layer which reveals the typical color of newly formed cork cells. The pycnial spots, whether young or old, are valuable diagnostic characters, because they often make possible the detection of infected trees before æcia are produced—that is, in time to destroy such trees before æciospores can spread the disease.

ÆCIA

Æcia appear in April, May, and June. Very often the whole area on which æcia can normally develop in a given season is covered with closely crowded sori pushing their way through widening cracks in the bark. By the middle of May the peridia are usually broken and the spores escape in orange-yellow pollen-like showers. Spore production continues for some time after the æcia open. Young æcia are easily obtainable at the edge of the æcial area where their presence is indicated by a thin yellow line just beneath the outermost layers of the bark cells. The hyphæ contributing to the formation of the æcium are aggregated into a mycelium, which is clearly made up of elements running tangentially among the host cells at a depth of 6 to 10 cells below the peridern layer. By their continued growth these hyphæ force the host cells apart, so that the latter become isolated and embedded in a matrix of tangled mycelium (Pl. 50, B). This separation may extend to a depth of 15 to 20 cells in the cortex. About 6 to 8 cells below the periderm layer a mass of pseudoparenchyma is formed by the packing together of the hyphæ. In the pseudoparenchyma a layer of fertile cells becomes discernible by their denser protoplasmic content (Pl. 50, A, *b;* 54, B, *fc*).

The fertile cells cut off rows of sterile cells (Pl. 54, B, *sc*), 6 to 12 cells long, which may increase in size at first, but which later degenerate to make

room for the developing æciospore chains. These sterile cells correspond to the buffer cells of Olive (*41*) and Fromme (*14, 15*). The cells of the fertile layer, which are somewhat larger than those of the vegetative mycelium, may fuse in pairs, the fusion beginning in the center of the sorus and proceeding centrifugally toward the periphery, as is normally the case in the æcium and its analogs. As a result of the fusion and union of the cell contents of the contributing cells a large cell is formed, the basal cell (Pl. 58, I, J), which will give rise acropetally, by repeated division, to the æciospore chain. The fusion seems to be complete as far as the cells are concerned, and evidently takes place rapidly, as there is only occasional evidence even in very young æcia of remaining parts of the cell walls which originally separated them (Pl. 58, I). The two cells are not always at the same stage of development, as one of the cells is often shorter or at a lower level than the other (Pl. 58, J). However, there is little ground for considering the two cells as different in kind. The essential process is the cell fusion and consequent association of the nuclei to form the dikaryon [1].

Fusion of more than two cells also occurs, trinucleate and tetranuclaete basal cells being quite common. Æciospore chains arising from such basal cells may contain the same number of nuclei as the basal cells, just as in æcia of other genera. Trinucleate basal cells are very numerous in young sori and at the edge of older ones, but the number of trinucleate æciospore chains is considerably less than one would be led to expect from the number of the trinucleate basal cells. There is a possibility that the extra nucleus frequently degenerates, evidence of this being occasionally seen. This point will be discussed briefly later on. Multiple cell fusions of a more complex character are also common (Pl. 58, K, L) recalling Olive's (*41*) and Fromme's (*14*) observations. The nuclei and cytoplasm of a number of adjacent cells, not only those in the upper row of the fertile layer but also others considerably below that level, become associated in one large irregular cell (Pl. 58, L). What happens to these large cells is not clear. The probability that the multiple fusion cells may give rise to a number of spore chains is reservedly suggested here.

FORMATION OF PERIDIAL CELLS AND ÆCIOSPORES

The basal cell divides into an upper part, the æciospore initial cell, and a lower part, potentially equivalent to the primary basal cell (Pl. 58, M). Each of the first few cells cut off from the basal cells normally divides into two cells of unequal size. The larger cells thus formed adhere more or less completely into a layer three to five cells thick which constitutes

[1] The term "dikaryon" is to be preferred to that of "synkaryon," the more common term, because of the earlier use of the latter name to designate the fusion nucleus resulting from the union of the male and female gamete nuclei by zoologists. Pavillard's (*43*) objection to synkaryon should be sustained, and Maire's (*33, 34*) suggestion that dikaryon be substituted for the older word has the virtue of correcting an error and supplying a distinctive term for the unique condition found in the rust sporophyte and the Basidiomycetes in general.

the peridium (Pl. 56, B); the smaller ones go to pieces. At the periphery of the sorus several adjacent basal cells cut off units which never become functional æciospores but which always form peridium. The small cells between these peridial cells indicate that the potential æciospore initial, although destined to become a peridial cell, regularly divides to form two cells homologous to an æciospore and an intercalary cell (Pl. 56, A). Thus, the multiple-layered peridium of *Cronartium ribicola* is formed in the same general manner as reported by Fromme (*15*) for the peridia of other deep-seated æcia. When first formed, the individual cells are subspherical to elliptical, and smooth-walled. As the sorus matures, their walls thicken and their outline changes according to their position in the peridium. All tend to become more or less polyhedral. The cells at the top of the sorus are usually more rounded than those at the sides, since the latter are elongated by the pull exerted on the sides as the developing spore chains force the central part of the peridium out. Therefore, the size of the cells varies widely (18 to 40 by 12 to 42 μ). The mature wall is 3 to 9 μ thick. The walls of the outermost cells are smooth or slightly granular, while the inner cell walls are studded with short tubercles which sometimes appear to mesh with those of the adjacent cells, perhaps contributing thereby to the strength of the peridium as a whole (Pl. 56, B). The cell contents of the peridial cells slowly degenerates until they become empty shells.

After the cells which form the peridium are abstricted from the basal cells, the æciospore initials are cut off. Each æciospore initial cell undergoes division into a larger upper cell, the æciospore, and a smaller lower cell, the intercalary cell (Pl. 58, W, M, *b*, X, Y, *c*, *d*). By the repeated divisions of the basal cell a row of alternating spores and intercalary cells is formed which constitutes the æciospore chain (Pl. 50, B; Pl. 58, Y). After the division of the æciospore initial cell, both resulting cells, the æciospore and intercalary cell, grow rapidly. The æciospore reaches its normal broadly elliptical shape when about three or four spores distant from the basal cell. The intercalary cells elongate, eventually becoming mere thin connecting elements between the æciospores in the chain, and finally disintegrate entirely. The spore wall thickens greatly when the spore has attained its full size. A thin space in the wall, suggesting the germ pore of other rust spores, is evident at the point of attachment of the æciospore and the intercalary cell below it (Pl. 58, Y, Z). This thinner place in the wall may persist even in the completely matured spore. It is comparable to similar phenomena in other rusts and does not normally function as a germ pore. The mature spore measures 18 to 21 by 20 to 26 μ. The æciospore wall seems to be made up of two parts, an endospore overlain by a somewhat thicker exospore. The latter is distinctly characteristic of æciospores of *Peridermium* spp. Part of it is cracked up into tubercles or warts, which makes it decidedly verrucose, and the rest of it is smooth (Pl. 58, Z). The smooth

area is more or less indefinite in extent at the basal end of the spore. Verrucose and smooth areas grade one into the other along the irregular line which separates them. The smooth area is fissured near its edge into blocks which become smaller and smaller until they approach the size of the tubercles of the verrucose area (Pl. 58, Z).

No completely satisfactory explanation of the manner in which this type of spore sculpturing arises has come to the writer's attention. The following theory is reservedly offered. The two walls of the spore are present when the spore is quite young, and both continue to grow and thicken up to a certain point or until the spore has nearly reached its full size. The outer wall hardens more rapidly than the inner one and in consequence becomes fissured irregularly as the still elastic inner wall continues to expand under the pressure of the growing spore content. The longer growth in size continues the more complete will be the fracturing process and the larger the verrucose area in proportion to the smooth area. Experimentally, the smooth area can be converted into verrucose area by soaking the spores in water. After several hours, if germination does not take place in the meantime, the spore absorbs water enough to cause it to increase appreciably in size. The tubercles of the verrucose area become free from their attachment to the inner wall and float around in the water. The smooth area, under the expansion pressure exerted as the inner wall swells, cracks and fissures until it becomes irregularly verrucose, approaching the conditions found on the normal verrucose area of the mature spore. The process of fissuring can be watched quite easily. The experiment at least suggests the manner in which a type of spore sculpture so oddly irregular could arise.

The spore wall on the smooth area has been heretofore considered to be thicker in section than the verrucose area. Examination of complete sections of the spores shows that this is not always the case. In fact, it is only in occasional instances that it holds true. As a rule, there is no appreciable difference in the thickness from the inner edge of the wall to the outer tip of any given tubercle and the thickness from the inner edge of the wall to the outer edge of the smooth area. When dealing with whole spores, refraction phenomena increase the difficulties in measuring the true thickness of a curving wall of the type presented in the smooth area of the æciospore of *Cronartium ribicola* and may account in part for the misinterpretation of the actual condition.

GERMINATION OF ÆCIOSPORES

Germination of the æciospores may take place rapidly under favorable conditions, but as Maire (*32*) aptly puts it "*la germination des ecidiospores * * * est parfois très capricieuse.*" The work of Spaulding and his assistants has shown that spores which would not germinate at all in hanging-drop cultures, on a water film, or on moist filter paper, either at room temperature or in the ice box, or at room temperature after cooling

in the ice box, were perfectly capable of producing infection on species of Ribes. These results confirm those of Klebahn (25), who found that æciospores which would not germinate in water did germinate very rapidly on leaves of *Ribes* spp. and only less rapidly but still abundantly on a gelatinous decoction of leaves of *Ribes* spp. These experiments suggest the probability that some direct chemotactic stimulus is exerted by the leaves of *Ribes* spp. on the æciospores. Klebahn has pointed out that there is considerable difference between the faculty for germination, as determined by artificial cultures, and the faculty for infection. Spaulding and his assistants have further determined that æciospores frequently germinate—even then only a relatively low percentage of fresh spores do so—more readily after cooling in the ice box than at room temperature, and that sometimes they have to remain in the ice box to secure germination. Too much water is often as inimical to germination as too little. A single spore may produce one to several germ tubes (Pl. 59, A), which attain considerable growth in artificial cultures. Where the germ tube passes through the heavy exospore it is constricted as shown in Plate 59, B. The tubes branch freely. The protoplasm is densest at the advancing tips of the hyphæ.

INFECTION OF RIBES SPP. AND MYCELIUM IN THE LEAF

Whether the germ tubes have the power to pierce the upper epidermis of the leaf of *Ribes* spp. or must always come to rest in a favorable position on the lower epidermis in order to cause infection is not definitely known. All the evidence gathered from the examination of artificially inoculated leaves points to the conclusion that infection occurs normally as a result of the germination of the æciospore on the lower surface of the leaf and the subsequent passage of the germ tube through a stoma. No evidence either of any break in the upper or lower epidermal cells, or of the remnants of any hypha passing through them, has been discovered. Furthermore, the mycelium is always abundant in the air chambers adjacent to the stomata, even in very young infections, and occasional remnants of spores and hyphæ near and in the stomata point to the stomata as the avenue of infection.

The first indication of infection in the leaf of species of Ribes is often indicated by the paling of the infected areas. Sections of such areas show that the mycelium has spread in the intercellular spaces and air chambers of the mesophyll. Haustoria (Pl. 59, D) enter all types of the leaf cells, with the possible exception of the xylem elements of the bundles, although they are comparatively rare in the epidermal cells. The cells of the mycelium and the haustoria are binucleate (Pl. 59, C, D). Generally there is a much smaller relative amount of mycelium in the leaf tissue than among the same number of host cells of the pine. The loose structure of the mesophyll allows plenty of room for the hyphæ to grow without severe crowding of the host cells. In fact, the hyphæ are aggregated only at the time of production of uredinia or telia.

UREDINIA

The development of the uredinium is illustrated in Plate 51, A, B, and 55, A–C, which form a series from the very beginning of the formation of the sorus to its maturity. When the uredinium starts to form, the fungus cells may be found aggregated in groups in some large air space, generally near a stoma. Certain of the cells in each group become oriented with their long axis more or less at right angles to the epidermis, against which they are closely appressed (Pl. 55, A). These cells are functionally equivalent to the basal cells of the mature sorus, in that from them arise the cells which go to make up the peridium, and the first urediniospores. The first division of any one of the vertically elongated cells results in the formation of a cell which adheres to its neighboring homologous cells to make the peridium (Pl. 55, B, *a*). In the second division the first urediniospore initial cell, or its equivalent, is cut off. This divides immediately to form the first urediniospore and stalk cell or their equivalents. The position of the cells referred to, as they appear in a young uredinium, are illustrated in Plate 55, B, in which *a* is the peridial cell, *b* the young urediniospore or its homolog, *c* the stalk cell or its homolog, and *d* the basal cell. It will be noted that the arrangement of the cells suggests that the sorus is made up of a compact aggregation of vertical rows of cells. This arrangement is temporary for the middle region of the sorus, but permanent for the circumference of the sorus. Plate 55, B, which represents a median section from a young uredinium, can be duplicated by taking sections through the edge of any mature sorus. This fact should be kept clearly in mind in any discussion of the structure of the uredinium.

The first urediniospores mature in the middle of the sorus (Pl. 51, B, *b*). Their formation and method of growth corresponds closely with the production of normal stalked urediniospores in other rusts. To repeat the process suggested above: The basal cell undergoes division (Pl. 55, C, *c;* 59, E–I). The upper cell is the urediniospore initial (Pl. 59, J, L); the lower cell is potentially the equivalent of the original basal cell. The urediniospore initial now divides (Pl. 59, K, L, M) to form a larger upper cell, the urediniospore, and a smaller lower cell, the stalk cell (Pl. 51, B, *b*, *c;* 55, C, *d*). While this process is going on, the layer of cells constituting the peridium gradually separates from the underlying urediniospores along the line between the cells marked "*a*" and "*b*" in Plate 55, A. The figures in Plate 51, A and B, represent steps in the process leading up to conditions shown in Plate 55, C. As the urediniospores grow, the peridium (Pl. 51, A, *a;* B, *a*) is forced up into a dome. The individual peridial cells lose the regular shape and outline shown in Plate 59, B, *a*, and become irregularly compressed or obliquely flattened. The growing urediniospores develop pressure against the epidermal cells, which flattens them out and finally causes them to be torn apart (Cf. Pl. 51, A, B, with Pl. 55, C). The break in the epidermis frequently

comes at a stoma, sometimes extending from one stoma to another; but the break in the peridium, when it finally occurs, is confined more or less definitely to the top of the dome. Usually the peridial cells around the break are irregularly thickened (Pl. 55, C). The orange-yellow urediniospores work their way out through the opening, sticking more or less closely to each other to form a spore crown on the top of the peridium. The basal cells continue to cut off urediniospore initials by a process similar to that involved in the formation of the primary initials, which has been described above. The secondary urediniospore initial may be formed alongside the base of the stalk of the first-formed urediniospore before the latter has reached maturity (Pl. 55, C, *c;* 59, N). The secondary initial then divides to form a urediniospore and stalk cell, in the same way as the primary initials. Meanwhile the stalk cell of the primary urediniospore elongates, withers, and goes to pieces. While no more than two spores have been found in connection with a single basal cell, each basal cell must frequently give rise to several spores, to judge from the number produced in a single sorus. The size of the mature spore (Pl. 59, P) is 10 to 20 by 19 to 45 μ.

The spore-bearing basal cells are confined to the middle part of the sorus. In figure C of Plate 55 it will be noted that on either side of the group of spores there is a group of parenchyma-like cells (*g*) made up of units which are arranged in more or less vertical rows. The cells of any individual row of four cells may be homologized with the cells lettered *a*, *b*, *c*, and *d* in figure B of Plate 55. This parenchyma-like tissue forms an encircling bank of cells which completely surrounds the mature uredinium. In this tissue the row arrangement of the cells shown in figure B persists, although the cells themselves lose their contents and become practically dead by the time the basal cells in the middle of the sorus are actively forming urediniospores.

The uredinium seems to be limited more definitely than either the pycnium or æcium in its ability to extend in a centrifugal direction. Its extent is predetermined, much more exactly than in the case of the two other sori, by the amount of massed mycelium from which it arises. Both pycnia and æcia originate in tissue which is comparatively full of mycelium before they start to develop. In the leaf of species of Ribes there is rarely any massing of the hyphæ to form the packed mycelium so common in the pine host, and the leaf cells are only occasionally distorted except in the immediate vicinity of the uredinia and telia.

GERMINATION OF THE UREDINIOSPORES

Urediniospores exhibit the same irregularity in germinating in artificial cultures as do the æciospores. Even when they are dusted over the surface of young, fresh, moistened leaves and placed in a damp chamber they may or may not germinate in large numbers. On a water film they absorb water and swell considerably. The cytoplasm becomes vacuo-

late. The germ tube passes through the exospore without the aid of a germ pore, assuming at first the shape of a more or less swollen vesicle (Pl. 59, Q). This lengthens rapidly into the young germ tube (Pl. 59, R), into which pass the contents of the spore. The endochrome and protoplasm are densest at the tips of the tube and in its branches. Duff (*12*) has shown that change of temperature stimulates the urediniospores to germinate and that light, especially ultra-violet light, may completely inhibit germination. This action of light may have a direct bearing on the apparent failure of the spores to cause infection at long distances from their source. All the evidence at hand points to the conclusion that the germ tube causes infection by passing through the stomata on the lower surface of the leaves. However, the tube may extend some distance over the leaf surface before actually entering a stoma, in such cases passing directly over stomata in its path. Urediniospores may retain their viability for some weeks. The uredinium is the repeating sorus in the life cycle of *Cronartium ribicola* (fig. 1), and the spores in succeeding generations infect leaves of *Ribes* spp. until late summer.

TELIA

Telial columns arise either from old uredinia or as entirely new and separate entities. They appear later than the uredinia and are more common in late summer. In the fall they are usually the predominant spore generation present. In the greenhouse they are produced throughout the year. On a given infected area the columns may occupy the central part where the uredinia were first produced, surrounded by a narrow peripheral region bearing the most recently formed uredinia. The development of the telial column is the same whether it is from an old uredinium or in a new sorus and, as the latter is probably the most common occurrence, it will be described. The massing of the hyphæ, formation of the peridium, and the parenchyma-like cells which surround the spore-bearing part of the sorus, and the orientation of the basal cells proceed exactly as in the uredinium. It is impossible to tell very young uredinia and telia apart. The binucleate basal cells undergo division (Pl. 59, S, T, U, V, W) in the same manner as in the case of the uredinium, but the cells cut off do not divide as in the other sori described. Instead they lengthen out and become teliospores. Each basal cell cuts off a vertical row of spores (Pl. 52, A, B), the central cells of the sorus producing spores slightly ahead of the cells at the periphery, as in the æcium and uredinium. As the spore columns lengthen, the peridium is pushed up into a dome (Pl. 52, A), which later ruptures (Pl. 52, B) and goes to pieces. The spores lengthen and soon reach full size, at about which time they become provided with a substantial wall slightly thickened at the upper end (Pl. 57, A, B, C). The first spores cut off—those at the tip of the column—are more or less polyhedral (Pl. 52, B; 57, A); the other spores are typically broad spindle-shaped (Pl. 52, D;

57, B). They usually touch, but do not crowd one another. Adjacent to the points where one spore abuts on its neighbors there may be considerable space. Cross sections of the columns (Pl. 52, E) show that the spores are only very slightly angular as a result of mutual pressure. Although they vary widely, normal mature teliospores average approximately 16 by 42 μ, with a wall about 2 μ thick. They are uninucleate, as a result of the fusion of the two nuclei which are normally present in the young teliospores. The mature telial column is an aggregation of a number of vertical rows of mature teliospores (Pl. 52, C). The length and diameter of the columns vary greatly; they may attain a length of 2 mm. and an average width of approximately 100 μ. They are usually curved or semispiraled, a result apparently of unequal development of the spores of some of the rows. They vary from almost spherical to irregular ovoid or elliptical in cross sections. Occasionally abortive, nondeveloped spores are scattered throughout the length of the column (Pl. 52, C, D). Other abnormalities will be discussed briefly later.

GERMINATION OF TELIOSPORES AND PRODUCTION OF SPORIDIA

All of the spores in the telial column may germinate *in situ* (Pl. 56, C). The exospore pushes out at some point in the form of a rounded papilla, which ruptures and allows the extrusion of a stout germ tube—the young basidium or promycelium (Pl. 57, D). This reaches its full size in a few hours and then becomes divided into five cells (Pl. 57, R) From each of the four upper cells arises a stout sterigma, on the tip of which the sporidium swells to its full size (Pl. 57, S–V). When abstricted, the sporidium is almost exactly spherical, measuring approximately 8 to 10 μ in diameter. At one point on the thin wall is a tiny papilla-like swelling, which marks the point of attachment to the sterigma (Pl. 57, AA, EE, GG).

The germ tubes from the spores in the middle of the column work their way out through the intersporal spaces and then develop in the manner described above. If the teliospores germinate under water, the germ tube lengthens out into a narrowly spiraled or twisted hypha; in other words, promycelia do not develop unless they have access to the air. The color of the germinating column, a very pale pink, gives a distinctly characteristic appearance to the fungus at this stage.

GERMINATION OF SPORIDIA

The sporidia germinate in artificial culture by sprouting relatively stout germ tubes (Pl. 57, X, Y, Z, BB) which probably continue growth in the normal fashion under favorable environment. In many cases, however, the short germ tube swells at its tip on reaching a length of several microns and the swelling becomes a secondary sporidium, apparently exactly similar to the primary one (Pl. 57, CC, DD). Sappin-Trouffy (51) has figured the same phenomenon for the sporidia of *Cronartium flaccidum*

Alb. and Schwein. on *Peonia officinalis*. It is apparently a common occurrence. Just how the sporidia reach the pine is not known, but it may be assumed that they are usually air borne. Infection of the pine host follows their germination under favorable circumstances on young pine shoots.

CYTOLOGY

PREVIOUS INVESTIGATION

Our knowledge of the cytology of the genus Cronartium is very fragmentary; in fact, no reference has been found to the nuclear phenomena accompanying the formation of any bark-inhabiting æcium of this group. Poirault and Raciborski (*44*) figure silhouettes of the nuclear division, as they interpreted it in the formation of the æciospores of the form known to them as *Peridermium pini acicolum*.[1] Sappin-Trouffy (*51*) gives a fair diagram of this acicolous type of æcium and the nuclear division at its base. His diagram of the telial column of *Cronartium flaccidum* indicates the phenomena accompanying the production of teliospores, though the figures are too minute to be more than suggestions of the actual conditions. He regards the processes as closely similar to the nuclear phenomena in other rusts, in which he is quite correct. Aside from the work of these authors, nothing beyond incidental mention of the cytology of the genus Cronartium has come to the writer's attention.

It is hardly necessary to give an extended resumé of the work of previous investigators on the cytology of the rusts, on account of the excellent reviews which have been published by Blackman (*2*), Christman (*4, 5, 6*), Fromme (*14, 15*), Maire (*32*), Guilliermond (*16*), and Ramsbottom (*46*). Moreau (*36, 37, 39*) revives the older view that there are but two chromosomes in the rust nucleus, which hardly seems tenable, and establishes the presence of centrosomes in the resting nuclei (*36*). This subject will receive further consideration later. Fromme's (*15*) recent paper on the morphology and cytology of the æcium clears up the matter of the formation of the deeper seated sori and establishes the similarity of spore formation in the cæoma and æcium. The life history of a complete rust is divided into two stages: the gametophyte, with uninucleate cells, and the sporophyte, with binucleate cells. The gametophyte begins with the reduction division, or its equivalent, in the promycelium and continues up to the fusion of the gamete cells at the base of the æcium, which initiates the dikaryon. The sporophyte begins with the inception of the dikaryon and continues up to the reduction division, or its equivalent, in the promycelium. The association of the nuclei in the basal cells of the æcium is regarded as the equivalent of a true fertilization, and the fusion of the two nuclei in the young teliospore as the completion of the process necessary for a mixing of the chromatin elements previous to reduction (*31*). The nuclear divisions are true mitotic divisions, accom-

[1] Vuillemin (*62*) also reports some of the phenomena accompanying æciospore formation in *Peridermium pini*.

panied by centrosomes or their equivalent, true spindles, and chromosomes. The character of the latter is still unsettled. In the dikaryon both nuclei divide simultaneously in conjugate division.

The chief differences of opinion have been in regard to the nature of the two cells which fuse to form the basal cells and in regard to the sterile cells. Blackman (*2*) held that the two basal cells were unequal, either in size or time of development, and that one, the larger, received the nucleus of the other, the smaller, by a process of nuclear migration through a comparatively small pore. After Christman's paper (*4*) announcing the fusion of two equal cells by the complete absorption of their appressed walls, Blackman and Fraser (*3*) investigated a number of forms and stated that the dikaryon might arise (a) through the process of nuclear migration from one cell to another, as Blackman first reported, or (b) by a similar nuclear migration from one vegetative cell to another below the fertile layer, or (c) by the process described by Christman. Most of the evidence since brought to view has supported Christman's theory. Olive (*41*) has pointed out that the manner of fusion is not of great importance, inasmuch as the conjugating cells are not definitely organized as male and female organs; for the essential result of the fusion is the establishment of a dikaryon by the association of two nuclei and their accompanying cytoplasm, regardless of their individual origin.

The sterile cells cut off from the fertile cells before fusion have been regarded as homologous to the trichogyne of the Florideae (*2*); as buffer cells which protect the developing basal cells below (*15*, *41*); or as degenerate female gametes, which once were fertilized by the now nonfunctional pycniospores. According to this last theory, advanced by Moreau (*40*), the cells as a group constitute a preæcium (*preecide*) and the individuals preæciospores (*preecidiospores*). Fromme (*15*) has shown that the number of sterile cells varies considerably in different species and suggests that they are formed in response to the general conditions of environment under which the particular sorus happens to be developed.

In the following presentation of the cytological details in *Cronartium ribicola* the fusion nucleus in the mature teliospore will be taken as the starting point, inasmuch as it is the first stage in the nuclear history of the gametophyte generation. The nuclear processes accompanying the production of the different spore forms can then be described in logical cytological sequence and at the same time in chronological order.

NUCLEAR PHENOMENA IN FORMATION OF PROMYCELIUM AND PRODUCTION OF SPORIDIA

With the germination of the teliospores the morphological history of the gametophyte may be said to begin. Coincident with this germination, changes occur in the fusion nucleus. As the promycelium reaches

its full size the fusion nucleus, formed by the union of the two nuclei in the young teliospore, migrates from the teliospore into the promycelium and starts to divide (Pl. 57, E). The membrane becomes irregular and disappears (Pl. 57, F) at the same time that the chromatin granules increase in size. These granules become longer and more deeply-staining units, which form a tangled mass in the position occupied by the fusion nucleus when it moved out into the promycelium (Pl. 57, G). A definite spindle, with centrosomes at the poles, then develops. At the middle of the spindle the chromatin tangle becomes arranged in an equivalent of the equatorial plate stage. In the next stages the chromatin is in the form of separate units (Pl. 57, H, I), which can be clearly seen under favorable conditions and proper differentiation. Plate 57, I, shows 16 such units which apparently are equivalent to distinct chromosomes. They separate into two groups, and the elements of the groups migrate along the spindle to the corresponding poles (Pl. 57, J, K). Radiations from the region of the centrosomes are present, but not easily stained during metaphase (Pl. 57, I), and quite long and prominent throughout anaphase (Pl. 57, K) and at telophase (Pl. 57, L, M). They seem to be due to cytoplasmic condensation rather than of the nature of true astral rays. The two chromatin groups in late anaphase are condensed into irregularly lobed masses (Pl. 57, L, M) at the ends of a more or less curved strand of suspension fibers, recalling Blackman's figure 31, Plate 21, (*2*). The suspension fibers disappear, the daughter nuclei reorganize around the chromatin groups as centers, and a wall divides the young promycelium into two cells (Pl. 57, N). A second division follows immediately (Pl. 57, O, P, Q). The two pairs of granddaughter nuclei thus formed are then separated by cross walls (Pl. 57, R), and the 4-celled promycelium completed. As a rule, the empty basal portion of the promycelium is also cut off by a wall, so that the promycelium really has five cells, four active and one, the stalk, practically dead. Each nucleus is relatively small, with a definite nucleolus and a minute centrosome. The contents take the form of a fine granular network.

From each of the four active cells of the promycelium a stout sterigma is protruded (Pl. 57, S), on which a single spherical sporidium is formed (Pl. 57, V). The nucleus of the cell migrates through the sterigma (Pl. 57, U), taking on an irregular shape during the process, and then rounds up into its normal form (Pl. 57, AA). The division of this nucleus to form a binucleate sporidium (Pl. 57, GG) is quite common, and the karyokinetic figures are particularly striking. Early and late anaphase stages are represented in figures EE and FF, Plate 57. The nuclear behavior in the formation of secondary sporidia was not followed. In germinating sporidia the nucleus probably migrates into the germ tube and there divides, although the figures of this process were indefinite and unsatisfactory. Figure BB of Plate 57 illustrates a common condition which indicates that the nucleus is preparing to divide, at the same

time moving toward the germ tube. At present no data can be given on the young mycelium from the uninucleate or binucleate sporidia, and this comparison must remain impractical until the process of sporidial germination and infection of the pine can be more closely followed.

NUCLEAR PHENOMENA IN VEGETATIVE MYCELIUM IN PINUS STROBUS

The uninucleate mycelium produced in the bark of *Pinus strobus* following infection by the sporidia has already been described. The nuclei are completely organized, with a definite nucleolus, chromatin network, and membrane (Pl. 57, HH, II). The chromatin appears to be more or less definitely centered on one point on the nuclear membrane where the centrosome is located. This condition of the arrangement of the chromatin elements of the nucleus will be referred to as polarization, and will be discussed in connection with its appearance in other stages in the cytological cycle. Few cases of vegetative division have been observed in the vegetative cells, but the stages of the process which have been seen indicate that there is a typical rust spindle and that the division is comparable to the vegetative divisions described by Olive (*41*).

NUCLEAR PHENOMENA IN PYCNIUM

The nuclei in the pseudoparenchyma layer from which the short branches bearing the pycnial sporophores arise are quite similar to those of the vegetative mycelium in shape, size, and organization, but they are colored more intensely with hematoxylin and the violet of Flemming's triple stain. The nucleus of the pycnial sporophore is relatively large for the size of the sporophore (Pl. 58, A). In its resting state the chromatin is scattered throughout the whole nucleus in minute granules, which are rather difficult to stain clearly. Each nucleus has a definite centrosome indicated by the local condensation of the chromatin (Pl. 58, A, *a*). The pycniospore (Pl. 58, A, *b*), which swells to its full size on the tip of the sporophore, receives one of the daughter nuclei resulting from the division of the sporophore nucleus (Pl. 58, A, *d*). The exact details of the process were not definitely followed, on account of the small size of the pycniospores and the narrow passage from sporophore to spore. The mature subpyriform pycniospore has the typical structure of corresponding forms in other genera of the rusts. A relatively large nucleus is surrounded by a small amount of cytoplasm (Pl. 58, A, *b*).

NUCLEAR PHENOMENA IN ÆCIUM

In the layer of fertile cells and in the mycelium below this layer the nuclei are somewhat larger (Pl. 58, H) and more readily stainable than those of the deeper vegetative mycelium (Pl. 57, HH). All the nuclei in this region of the layer and below it exhibit the phenomena of polarization, although the fact may not be evident unless the profile view can be seen. The same condition persists in all other nuclei in or near the vari-

ous sori. Above the fertile layer a series of cells, the sterile cells (Pl. 58, G), arranged in more or less definite vertical rows, are cut off (Cf. Pl. 54, B, *sc*). Fromme (*14*) finds that the sterile cells in *Melampsora lini* (DC.) Tul. arise from the division of the first sterile cells; Moreau (*40*) states that in *Phragmidium subcorticium* they are formed by the division of the cells in the fertile layers. In *Cronartium ribicola* they seem to arise from the cells of the fertile layer (Pl. 58, F), but there is some evidence that the sterile cells themselves also divide occasionally. Each row contains from 6 to 12 cells, whose contents degenerate as the æciospores mature. The pressure of the peridium and the lengthening æciospore chains flatten the whole tissue of sterile cells against the overlying cortex cells.

CELL FUSION TO FORM BASAL CELLS

Fusion normally occurs between two adjacent cells of the fertile layer, which may or may not be at the same level (Pl. 58, I, J). The walls between the fusing cells appear to dissolve, leaving one large cell where there had been two. The basal cell thus formed is the initial cell of the dikaryon. The nuclei are comparatively large (cf. fig. G and I, Pl. 58). They exhibit the polarization phenomena referred to above, but their content is less easily stainable than in the vegetative nuclei below the fertile layer (cf. fig. H and I, Pl. 58), for at times the space between the nucleolus and the membrane seems almost empty (Pl. 58, Y, *a*). When the nuclei are located in dense cytoplasm toward the upper end of the basal cell, the lower end may be occupied by one or more large vacuoles (Pl. 58, Q). When fusion of more than two cells occurs, the nuclei usually are more irregularly placed (Pl. 58, K, L). The nuclei in these compound basal cells and in the multiple fusion cells are similar to those in the normal binucleate cell.

THE CONJUGATE DIVISION IN THE BASAL CELLS

The division of one of the nuclei of the basal cell will be described, it being understood that the companion nucleus undergoes the same changes at the same time; that is, that the division process is a conjugate division typical of the rusts. On the nuclear membrane is a body which stains deeply with hematoxylin—the centrosome (Pl. 58, I). From it in most cases there run strands of chromatin which are more dense than the other chromatin elements of the nucleus. The nucleus increases in size and the membrane bulges, except at the points where the chromatin strands touch it (Pl. 58, Y, *a*). At this stage the membrane becomes very thin and then disappears, fading first on the side away from the centrosome (Pl. 58, M, *a*). Meanwhile the chromatin elements group around or at one side of the nucleolus. Fine suspensors appear to connect the centrosome and the condensing chromatin mass. In the left nucleus in Pl. 58, M, *a*, the centrosome appears to have divided into two. Coincident with

the disappearance of the nuclear membrane the nucleolus is cast out (Pl. 58, N), after which it migrates off to some distance from the chromatin mass. A true spindle is then formed at the two ends of which are located the centrosomes (Pl. 58, O, P). The original centrosome was not seen to divide, but the conformation of the spindle in some early stages suggests the division of the centrosome to form two, which move apart and become oriented as the opposite poles of the spindle. The chromatin now appears in deeply stained sections as a convoluted dark mass at the middle of the spindle (Pl. 58, O). With better differentiation this mass resolves itself into a jumble of more or less rounded units, the chromosomes (Pl. 58, P). This stage corresponds to the equatorial plate stage of the metaphase. The chromosomes are now seen to separate and move toward the poles (Pl. 58, Q–S); they seem to flow along the outer surface of the spindle rather than to be drawn definitely apart by attraction fibers. Unless very carefully differentiated, the chromatin at this stage may appear to be a single knotted thread reaching from pole to pole. The chromosomes, however, do not fuse into one mass, but, as can be seen in favorable preparations, remain as separate units (Pl. 58, R). As they approach the poles the chromosomes apparently arrange themselves into two groups (Pl. 58, T) for each pole. The division of the centrosomes before the daughter nuclei are reorganized may explain the locus of the two groups, as suggested by Olive (*41*). At this late stage of anaphase the whole figure may resemble the silhouette dumbbell figures by earlier investigators of the subject. The two groups at each pole now become condensed to two deeply staining masses (Pl. 58, U). Fibrous connections between the daughter groups may still remain visible. The cast-out nucleolus has persisted in the cytoplasm up to this time, slowly becoming less dense and often decreasing in size, while the process described above has been taking place, but as the last spindle fibers disappear and the daughter nuclei begin to become reorganized it fades away, completely absorbed by the cytoplasm. The companion nucleus of the dikaryon, having divided simultaneously with the one described, has by this time given rise to two other daughter nuclei. The four reorganizing nuclei now move apart, two and two, the sister nuclei separating and moving in opposite directions. A wall now forms (Pl. 58, V, *a*), separating the upper pair from the lower, forming the æciospore initial and, below it, a new basal cell (Pl. 58, M), potentially equivalent to the primary basal cell.

STEPS IN FORMATION OF ÆCIOSPORE CHAIN

Division of the dikaryon in the æciospore initial follows the same process as that in the basal cell (Pl. 58, W, M, *b*, X). The wall which forms between the two pairs of nuclei divides the original initial cell into two parts, an upper cell which is the æciospore and a lower, which is the intercalary cell (Pl. 58, Y, *d*, *c*). Repeated division of the basal cell and

æciospore initials gives rise to a row of alternating æciospores and intercalary cells (Pl. 58, Y), as has been stated in the description of æciospore formation elsewhere in this paper. The nuclei of the æciospores soon become completely reorganized (Pl. 59, AA) and typical of the dikaryon in all its stages, but the nuclei of the intercalary cells slowly degenerate and disappear. A centrosome, the center for the polarization phenomena previously mentioned, can be differentiated by proper staining on the membrane of each nucleus.

NUCLEAR PHENOMENA IN UREDINIUM

The mycelium in leaves of species of Ribes has already been described. The nuclei at this stage of the dikaryon are only slightly less in diameter than that of the hyphæ, and they are therefore somewhat separated—not side by side as in the elements of the æciospore chain (Pl. 59, C). They do approach each other in the larger cells at the base of the uredinium and undergo conjugate division by a process apparently identical (Pl. 59, E–I) to that described for the basal cell and æcispore initial of the æcium. The same holds true for the division in the urediniospore initial (Pl. 59, J–M, N, *b*). The wall formed between the two pairs of daughter nuclei divides the initial into an upper, larger cell, the young urediniospore, and a lower, smaller cell, the young stalk cell, which rapidly elongates. The nuclei in the spore become organized similarly to those of the æciospore (Pl. 58, AA), while the stalk nuclei slowly degenerate (Pl. 59, N). The second and subsequent conjugate divisions in the basal cell (Pl. 59, O) are similar to the primary division, but the nuclei often are not so definitely placed side by side, an irregularity probably due to the tendency of the cytoplasm flowing into the second urediniospore initial to pull one of the nuclei along with it. As a rule, both in the basal cell of the æcial chain and in the basal cell of the uredinium at the time of the primary division, the nuclei, though they may commence to divide when located at different levels in the cell, become arranged side by side at metaphase. In the second division of the urediniospore basal cell this orientation may not take place, so that at telephase one of the daughter nuclei may be well up in the new urediniospore initial and its companion just passing into it; but on reorganization after the initial is cut off from the basal cell, the two nuclei take a position side by side in the typical manner. Their division and the subsequent cutting off of the stalk cell by a wall give rise to a second urediniospore. This process may be repeated several times, for the basal cell seems to retain its powers of division until the sorus dries up.

NUCLEAR PHENOMENA IN TELIUM

The telia are borne on the same mycelium that gives rise to uredinia. By an exactly similar process of conjugate division (Pl. 59, S–W) a cell is cut off from the basal cell of one of the telial unit columns; but this

cell does not divide again, being in itself the young teliospore (Pl. 59, X). The two nuclei appear to become fully reorganized (Pl. 59, Y). They then fuse immediately to produce the single large nucleus of the mature teliospore, during which process the nuclear membranes are absorbed at the points of contact, so that the nuclear contents are free to mix (Pl. 59, Z). The fusing contents round up and become surrounded by the reorganizing membrane. In Plate 59, Z, the two centrosomes and the two nucleoli are still visible. The process of the fusion of the latter was not observed. The fusion nucleus increases in size in a remarkable manner immediately after the union of the two contributing nuclei; and the enlargement may continue until the volume of the fusion nucleus is as much as four times the combined volumes of the two contributing nuclei. Its diameter at the time of maximum enlargement nearly equals the diameter of the teliospore. The chromatin at this stage stains very heavily and appears to be condensed into a heavy irregular spireme-like structure, in which the individual strands are frequently knotted (Pl. 59, AA). Holden and Harper (*21*), in discussing the fusion nucleus in the teliospore of *Coleosporium sonchi-arvensis* Lev. (*C. solidaginis* (Schw.) Thum.?), present evidence that the heavy skein breaks up into long pieces, which later split longitudinally into finer threadlike units. In *Cronartium ribicola* there certainly occurs a marked reduction in the thickness of the chromatin threads, but no process which could be interpreted as actual splitting was clearly seen. As the chromatin becomes more finely drawn it contracts into a more compact tangle, the nucleus shrinking meanwhile (Pl. 59, BB), and finally splits into granules (Pl. 59, CC). The nucleus at this stage is nearly spherical; the nucleolus is rather small, and is generally located near the membrane. The centrosome could not be differentiated in the fusion nucleus with any stain. So far as could be determined by careful examination, it does not reappear until the primary division in the promycelium and in the resting nuclei of the promycelium.

DISCUSSION OF CYTOLOGICAL PHENOMENA

The nuclear phenomena accompanying cell fusion and spore production in *Cronartium ribicola* clearly confirm the views generally held in regard to rust cytology. Fusion of the gamete cells in pairs in the bark-inhabitating æcium parallels closely similar phenomena in other types of æcia and analogous sori, while multicellular fusion is perhaps much more common than in other forms investigated. Fromme (*14*) has called attention to triple-cell fusions and the fact that cells below the fertile layer often contribute to the multinucleate fusion cells of *Melampsora lini*. The behavior of the fusion cells in *C. ribicola* shows that cells below those of the fertile layer are potential gametes. It is quite certain that the multiple fusions observed are regular occurrences in æcia of all sizes and shapes, whether on roots or stems. Dittschlag (*11*) and Hoffman

(*20*) both figure two æciospore chains rising from a large basal cell in *Puccinia falcariæ* and *Endophyllum sempervivi*, respectively, and there is some evidence that aeciospore chains often arise in like manner in *C. ribicola*. It is hardly possible, however, to compare the multiple fusion cells to the central placental cells reported by Richards (*49*). Inasmuch as the elements of the æciospore chains are generally binucleate, the number of polynucleate spores being relatively small, it must be concluded that either the extra nuclei so common in the basal cells degenerate or the complex basal cell gives off more than one binucleate spore chain.

The constant similarity in the process of congugate division in the basal cells of the æcium, uredinium, and telium suggests a definite stability of the nuclei of the dikaryon throughout its existence. The presence of the centrosome in the resting and dividing nuclei adds confirmation to the reports of this structure in rust nuclei as given by other writers. While it is impossible to make a definite statement from actual observation of the process, it seems perfectly evident that the centrosomes at each pole may divide, thus forming two loci for the chromatin groups approaching the poles—cf. Olive's figures 5, *a*, and 10, Plate 22 (*41*). The theory that these two groups of chromatin represent two simple chromosomes, the interpretation of the phenomenon apparently accepted by Mme. Moreau, does not appear tenable, unless it is assumed that these chromosomes are compound and that they break up into their components at metaphase and reunite at telophase. The observations of Holden and Harper (*21*), Blackman (*2*), Christman (*4*), and Olive (*41*) and the evidence presented in this paper establish the presence of more than two chromosomes or chromosome equivalents, possibly eight being the haploid number. Moreau's (*37*) figures indicate that her preparations were apparently not sufficiently differentiated to show all the details in the chromatin masses.

The polarization of the nuclei, so evident in nearly all of the resting stages, recalls the condition illustrated in Olive's figure 8, Plate 22 (*41*). The arrangement of the chromatin with a point on the membrane as a locus for the convergence of the strands is not due to fixation; for it is a constant phenomenon in nuclei killed and fixed under different conditions and with different reagents. The centrosome is apparently the center of attraction. Harper (*18*) has conclusively demonstrated similar phenomena in the Erysipheae. He has also called attention to the fact that the nuclei of these ascomycetes exhibit a definite regularity and stability with reference to the arrangement of the chromatin strands and the polarization throughout the cytological changes which he observed. He believes the chromosomes retain their individuality to a marked degree in all the nuclear processes. The observations on the nuclear structure in *Cronartium ribicola* herein presented suggests that

perhaps the conditions in the rust nucleus approach more nearly than has been supposed the conditions in the Ascomycetes and other fungi. The process of nuclear fusion in the young teliospore is comparable to the same process in other rusts. It seems reasonable to conclude that this fusion is the completion of the process, initiated at the time of cell fusion to form the dikaryon, which takes the place of normal fertilization, and that the actual fusing is a necessary preliminary to the mixing of the chromatin elements and the subsequent reduction division, as suggested by Maire (*31*) and others. Certainly the changes which take place in the nucleus after fusion suggest a complicated mingling and readjustment of the chromatin which would seem to justify such a view. It is not necessary to regard the fusion as a pseudo fertilization (*10*). There appears to be little reason for doubting that the first division in the promycelium is heterotypical (*38*), for it is unique and decidedly different from the second division which immediately follows. Arnaud (*1*) has compared the mitotic figures in *Capnodium meridionale* with those in *Coleosporium senecionis*. Wager's figure 84, Plate 19 (*63*), of the telophase in the dividing fusion nucleus in the sporangium of *Polyphagus euglenae* is very like similar stages in the primary division in the promycelium of *Cronartium ribicola*. It may be noted that the association of the nuclei in *P. euglenae* and their subsequent fusion in the sporangium are phenomena comparable to the nuclear conditions in the rust dikaryon and fusion in the teliospore.

ABNORMALITIES

Before coming to the general discussion and summary it is necessary to mention briefly certain abnormalities commonly met in the different types of sori. Æcia sometimes occur with reversed polarity in part of the sorus, where the spores are produced on æcial chains which grow toward the center of the tree, usually into a resin duct. This change in the direction of growth is probably to be explained by the fact that the developing chains followed the line of least resistance, in this case into the adjacent resin duct, instead of against the overlying host cells. Æcia also often develop on the roots, under several inches of leaves and loam. Their structure appears to be normal, but their environment is hardly advantageous. Double pycnial layers (Pl. 50, C, *a*, a_1) are not uncommon. In a short note, Posey, Gravatt, and Colley (*45*) have reported the finding of uredinia on the stems of *Ribes hirtellum* Michx. In the cortex of infected stems of this species internal uredinia (Pl. 51, C) with normal and reversed polarity were formed in abundance. Internal telia, produced in the pith and cortex of the petioles of *Ribes* sp.,[1] have been described in a previous paper (*8*). In such abnormal

[1] *Ribes roezli* was the name given in the original article, but the species determination was probably incorrect. As the species has not fruited, accurate determination has been impossible.

sori, typical column development is sometimes completely inhibited by the pressure of the surrounding host tissue. All internal sori are to be regarded as teratological phenomena of no special morphological significance. Uredinia and telia on the petiole are common; occasionally they develop on the upper surfaces of infected leaves.

GENERAL DISCUSSION

The writer's observations on the mycelium of *Cronartium ribicola* in the bark of *Pinus strobus* confirm the conclusions of Klebahn (*25*) in regard to the course of the hyphæ of the parasite and add considerable new data on the inter relations of the host and parasite cells. Hartig's paper (*19*) on *Peridermium pini* and Wolff's article (*64*) on the same fungus, which appeared shortly after Hartig's and in some places is almost a direct copy of Hartig, are the only papers which have come to the writer's attention which describe and illustrate the morphology and parasitic relations of a bark-inhabiting rust and its pine host. Hartig believes that the swelling of the bark of the host is due to the fact that the cortex and phloem cells are forced apart by the abundant intercellular mycelium of the parasite. This has been shown to be the case with the hypertrophy produced by *C. ribicola* in the bark of *P. strobus*. Observations on the bark of *P. parviflora* Sieb. and Zucc. infected with the same fungus show that the swelling is produced in the same way as it is in *P. strobus*. The same statement holds true for the swelling caused by *C. comptoniae* Arth. in the bark of *P. sylvestris* L., and *P. ponderosa* Laws. Neither *C. ribicola* nor *C. comptoniae* are gall-forming rusts. Their mycelium is confined almost entirely to the region of the cortex and phloem cells of their pine hosts, although the hyphæ do enter the wood along the rays, as Tubeuf (*58*) has reported for *Peridermium pini*, and occasionally work their way in between tracheids. These hyphæ have been traced in the case of *C. ribicola* to a depth of three annual rings, counting in from the cambium. In all probability it will be found that the mycelium may be found in the ray cells of the annual ring laid down at about the time infection took place, although it may not remain active after the ray cells die. While the presence of the hyphæ in a given annual ring may not mean that the pine was infected during the year this annual ring was laid down, because there must be some growth along the ray cells toward the center of the tree, it will establish roughly the date of infection. The similarity which evidently exists between the morphology and method of parasitism of *C. ribicola* and *C. comptoniae* suggests that a close agreement will probably be found among all non-gall-forming caulicolous Peridermia.

The actual injury to the pine host cells from the irritation caused by the invading mycelium is apparently very slight. The cells pierced by

haustoria continue to remain alive and apparently active in spite of haustoria. As has been pointed out above, these haustoria become invested with a sheath which may render them very ineffective absorbing organs, in which case they might become simple mechanical irritants in the cell content, no more detrimental to cell activity than crystals. The denting of the host nucleus by the tips of the haustoria does not seem to injure the nucleus except to alter its shape. There is no evidence of increased cell division reported by Reynolds (*48*) for many plants as a direct result of a parasite's action, or of nuclear migrations like those figured by Schürhoff (*52*). Storage starch is usually present in excess, but this phenomenon has been shown by Halsted (*17*) to be a general condition in and around areas infected with fungus parasites. This starch is not completely used up by the fungus, for many grains remain in the old dead cells after the cells are completely dried out. The cells just beneath the pycnial layer and in the region of the young æcium (Pl. 52, B) contain normal grains as well as decomposition products. Excess starch production is probably due to a lack of balance in the physiological processes in the host cells, and the fungus may, of course, contribute to the unbalancing; but other environmental factors which are little understood result in excess starch production in trees which appear to be perfectly normal in other respects.

The wood laid down in the annual rings under infected bark is much less than in healthy trees, but the tracheids are apparently normal in everything except number. The presence of the hyphæ in the tracheids has no appreciable influence on their form; neither are the characters of the ray cells perceptibly changed.

Cronartium ribicola may be the primary cause of the death of a young tree. However, the swelling of the bark is not in itself a serious hindrance to conduction in the phloem. The actual severe injury occurs when the æcia form and burst through the outer bark; for the æcial cracks thus formed allow the inner bark cells to dry out and die. This results in the breaking of resin canals and the consequent exudation of resin in large quantities. The girdling of the tree is due to these two causes working together—namely, the cracking and drying of the bark, and the impregnation of the whole cortex and phloem in the cracked area with resin. Complete stoppage of the conducting elements of the phloem results. Seedlings and young trees may succumb to the attack of the fungus almost as soon as the first æcia appear; but with older trees death is sometimes delayed for a number of years. It depends, of course, on how complete a girdle has been effected. The part played by secondary fungi acting in conjunction with *C. ribicola* to make a girdle complete is very important. It will be recognized at once that the cracking of the bark at the time the æcia are formed is a source of danger to the trees not only by exposing the inner bark cells to the air, but also by providing an avenue of entrance for secondary

parasitic fungi, or saprophytic fungi, and insects, which hasten the decomposition of the weakened susceptible tissue. These secondary fungi may also gain entrance through the pycnial spots. Rathay (*47*) has shown that the pycnia of many rusts are visited by insects, apparently attracted by the sweet drops. Both pycnia and æcia in *C. ribicola* may be eaten out by various insects, and unquestionably these insects bear on their bodies or legs viable spores of fungi capable of growing in the bark tissue. In many instances these fungi are so rapid in their growth that they overrun the infected area and completely suppress the rust, so that it never forms æcia. In such cases, the bark shrinks and the infected stem is actually constricted, and the girdle thus formed is often more quickly effective than in the cases where rust works alone.

In leaves of *Ribes* spp. the mycelium sometimes causes the death of isolated infected spots; but in other cases the hyphæ penetrate to all parts of the tissue without causing death of the cells, and without producing hypertrophy. The large spaces among the mesophyll cells and the fact that the hyphæ rarely form solid mycelial masses in leaves of *Ribes* spp. probably help to explain the lack of hypertrophy and destructive effect. When defoliation occurs during the course of severe epidemics of the rust, there is, of course, a consequent poor crop of berries. The variation in the effect of the parasite on the different species of Ribes is a subject which must have separate treatment and therefore can not be considered fully at this time.

It will probably be found on further investigation that a close agreement exists among caulicolous Peridermia with respect to the structure of the pycnium. The external appearance of the pycnium, or of the thin layer of tissue overlying it, will, however, be found to vary according to the outer bark texture of the host. In the case of *Pinus parviflora* the exterior appearance of the pycnium is almost identical with the pycnium of *P. strobus*. The value of the pycnial spots as diagnostic characters has been briefly outlined in an earlier short note (*9*), and referred to above in connection with the discussion of the formation of the pycnium and pycniospores. Hartig (*19, Taf. 4, fig. 7, b*) was one of the first observers to call attention to the pycnial spots on the bark of *P. strobus*, although he at that time thought the fungus on this pine was identical with *Peridermium pini*. Wolff (*64*) copied the same figure in his paper on the latter fungus. Kirchner and Boltshauser (*22*) in Plate 15 of their atlas show what are evidently three pycnial spots, but they do not definitely refer to them in their description of the figures. The ability to recognize the pycnial spots of *Cronartium ribicola* is almost absolutely essential in the field study of control methods.

The description of the formation of the æcium given above emphasizes the remarkable agreement which exists in the fundamental processes involved in the production of æciospores in the rusts. The figures of Hartig (*19*), Wolff (*64*), and Sappin-Trouffy (*51, fig. 65*) on the æcium of

Peridermium pini are not complete nor detailed enough to enable one to compare directly the structure of the acicolous and caulicolous æcia with the structure of the æcium of *Cronartium ribicola*. It is evident, however, that the leaf and stem types are quite similar. Study of the expansive æcia of *C. occidentale* Hedgc., Bethel, and Hunt may reveal some interesting morphological variations.

The details given for the formation of the multilayered peridium are apparently the first published record of the origin of this structure. Emphasis should perhaps also be placed on the constant and normal occurrence of multinucleate cells at the base of the æcium, a phenomenon which has been discussed elsewhere, by suggesting that in deep-seated æcia of caulicolous Peridermia the æciospore chains may be found to arise more often from these placenta-like cells than from basal cells arising as a result of the fusion of only two cells in the fertile layer. Although recent investigators—for example, Kurssanow (*27*)—have confirmed the results of Christman (*4*) and other writers with regard to the origin of the basal cell as a result of the fusion of two adjacent fertile cells, it yet remains a question whether this method is constant or whether certain variations in the formation of the basal cells are to be expected in deep-seated æcia.

Ludwig and Rees (*28*) in a recent article report some details of the structure of the uredinium of *Pucciniastrum agrimoniae* (Schw.) Tranz. Their figure would serve very well for a figure of the young uredinium of *Cronartium ribicola* (cf. Pl. 55, B). In the description of the uredinium of the latter it has been shown that the peridium is formed by the coalescence of cells which are cut off from certain cells that are analagous to the basal cells of the sorus. These cells also cut off urediniospore initials which then divide into urediniospores and stalk cells. In the young sorus these four cells—that is, the basal cell, the stalk cell, the urediniospore, and the peridial cell—form what looks like a chain of cells. As the spores and their stalks mature, the row arrangement is lost in the middle of the sorus, but persists at the circumference. This fact places the conclusion of Ludwig and Rees that the urediniospores of *P. agrimoniæ* are borne in chains, under suspicion. Personal investigation by the writer into the structure of the uredinium in this species of Pucciniastrum shows that the method of formation of the urediniospores in *P. agrimoniæ* and in *C. ribicola* is practically identical, and that therefore the spores in the uredinium of the former are not borne in chains but on stalks. In the case of *P. agrimoniae* the stalks are sometimes quite short and the basal cells from which they arise are much less conspicuous than they are in *C. ribicola*, but these differences are not important as far as the method of spore formation is concerned. The encircling bank of parenchyma-like cells surrounding the uredinium in *C. ribicola* is not found in *P. agrimoniae*. It is difficult to interpret Magnus's (*30*) figures of the uredinia of the Pucciniastrum group as to the exact morphology of the cells from

which the urediniospores arise, but his description of the manner in which the urediniospores in Pucciniastrum are borne is correct. Fischer's (*13*) figure of the uredinial peridium of *C. asclepiadeum* (Willd.) Fr. evidently represents the same type of peridium as is present in the uredinium of *C. ribicola*. The peridial cells surrounding the break in the top of the peridium in the latter fungus are thickened irregularly, but they do not often appear as conspicuous as the large cells figured by Fischer (*13*) in the case of the uredinial peridium in species of Pucciniastrum or by Ludwig and Rees (*28*) for *P. agrimoniae*.

In the discussion of the telium it has been shown that it originates in a way which makes it impossible to tell the young telium from the young uredinium. Under these circumstances it is most natural that a peridium should be found over the telium, as in the case of the young uredinium. The same holds true for the parenchyma-like bank of cells which encircle the base of the column. Both the peridium and the bank of encircling cells come into being before the sorus becomes differentiated into either uredinium or telium. With these facts clearly in view it may be reasonably safe to predict that a telial peridium, a structure which has apparently not been reported previously, will be found to be present in other species of the genus Cronartium. The manner of the germination of the teliospores, previously discussed, adds to our knowledge of the morphology and behavior of the spores in the telial column, at the same time confirming the observations of Tulasne (*61*) and Sappin-Trouffy (*51*) on other species.

This paper is offered as a contribution to our knowledge of the parasitism, morphology, and cytology of the rusts, and especially of the genus Cronartium. It is hoped that the data presented may prove valuable in stimulating further research on the interrelations of rust parasites and their hosts, which will, of course, involve more accurate study of the anatomy of the hosts and the modifications in the normal structure of the host tissues under the action of the parasites. The need for a comparative paper on the haustoria of the rusts scarcely requires emphasis. It will be interesting to compare the results of investigations on the cytology of other deep seated caulicolous æcia with those herein presented, especially with reference to the process of the formation of basal cells, the phenomena of polarization of the nuclei, and the centrosomes and chromosomes.

SUMMARY

(1) In the foregoing paper hitherto unpublished data on the morphology and cytology of *Cronartium ribicola* Fischer and the interrelations of the parasite and its hosts, *Pinus strobus* and *Ribes* spp., are presented and fully illustrated.

(2) The mycelium is more abundant in the *Pinus strobus* than in species of Ribes. In the former the hypæ force the cortex and phloem

cells apart and thus cause the swelling of the infected bark; in the latter there is rarely any marked aggregation of the hyphæ, except in the case of petiolar infection.

(3) Haustoria may penetrate practically every cell in the infected area in *Pinus strobus*. These haustoria are characteristic for *Cronartium ribicola* and their presence in the bark cells of the pine definitely determines the identity of the parasite. A sheath develops around each haustorium as it reaches maturity or old age. This sheath is apparently unlike any other sheath so far described in connection with the haustoria of the rusts.

(4) Haustoria are proportionately much less frequent in *Ribes* spp. than in *Pinus strobus* and much smaller in size. They are not enveloped in sheaths as in the latter.

(5) The morphology of the different sori is shown to be similar to the morphology in other full-cycle rusts.

(6) The structure of the spreading pycnial layer characteristic of caulicolous Peridermia is considered in detail.

(7) The development of the deep-seated æcium and the formation of its multilayered peridium is described.

(8) The formation of urediniospores is shown to follow the general plan in other uredinia where the spores are borne on stalks. The development of the uredinial peridium and the bank of parenchyma-like cells which surround the uredinium are described and figured for the first time.

(9) The telial column is shown to arise either from an old uredinium or as a separate entity and to be indistinguishable from a young uredinium in its very early stages. It is surrounded at the base with a bank of parenchyma-like cells and is provided with a peridium similar to the peridium of the uredinium. All of the spores of the telial column may germinate *in situ*. The production of sporidia is described in detail.

(10) The destructive effect on the pine host as a result of the attack of *Cronartium ribicola* varies. In young trees death may result quickly. In older trees it is of the nature of a primary injury, which prepares the way for the drying out of the infected bark and the entrance of secondary fungi and insects which complete the destruction initiated by the parasite.

(11) The effect on Ribes varies with the species attacked. It may result in early defoliation and a consequent poor crop, but in general it is not serious on this host.

(12) The cytological phenomena presented herein establish the similarity of the nuclear processes in the genus Cronartium and other full-cycle rusts.

(13) The resting nuclei in all cases, with the exception of the mature fusion nucleus in the teliospore, have a deeply staining spot on the

membrane called the centrosome. This body is apparently the center for the more or less definite convergence and concentration of the chromatin strands referred to as polarization.

(14) The presence of large, irregular, multinucleate cells at the base of the æcium is a regular phenomenon and not to be regarded as an abnormal condition. Probably more than one æciospore chain may arise from such cells.

(15) There is a remarkable constancy in the process of conjugate division and stability in the nuclear structure throughout the dikaryon. In the dividing nuclei the centrosomes are visible as deeply staining dots at the poles of the spindle. The number of chromosomes is certainly more than two. Possibly eight is the haploid number.

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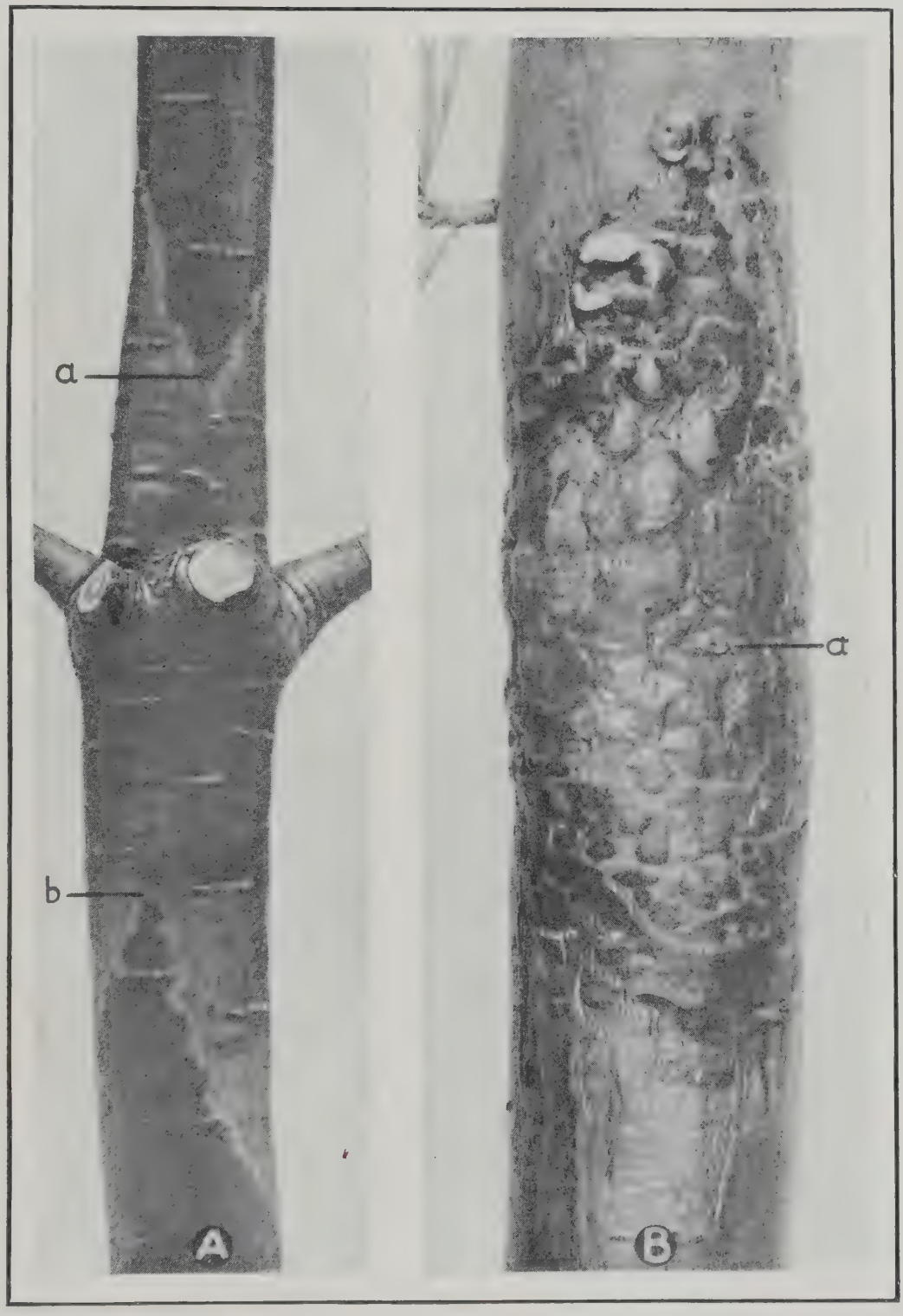
PLATE 48

Cronartium ribicola on *Pinus strobus:*

A.—This figure illustrates the etiolated condition of the bark in the case of a comparatively young nodal infection. The node lies between 4- and 5-year-old wood. The infection originated on the opposite side of the stem to that shown in the photograph. Note the irregular margin of the etiolated area. The mycelium, starting in opposite directions from the infection point, has now completely encircled the stem. The junction point of the encircling hyphæ is along the line *ab*. Natural size.

B.—This figure illustrates an internodal infection, somewhat older than that shown in figure A, in which the infection apparently originated at the base of the leaf fascicle (*a*). The darker patches on the pronounced canker area are pycnial spots. Æcia are formed under the bark all over the canker and are beginning to break out toward the upper end. Note the characteristic shape of the young canker. Natural size.


a
b
A
a
B

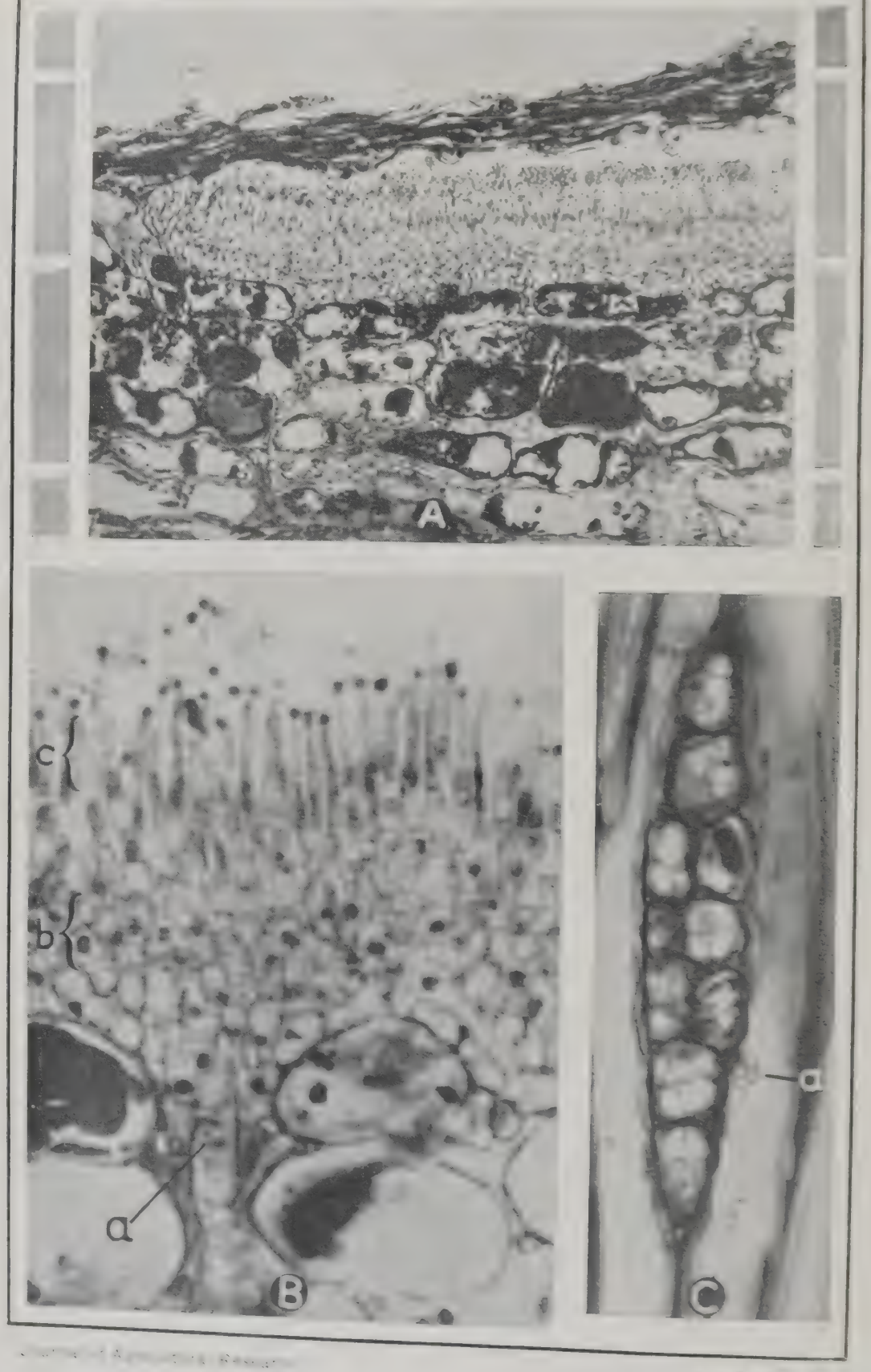

A
c
b
a
B
a
C

PLATE 49

Cronartium ribicola:

A.—The edge of a pycnium in section. Note the position of the pycnium with reference to the overlying periderm and underlying cells of the outer cortex. × 250.

B.—Part of the same section showing the general relation of the elements which go to make up the sorus and their relation to the host cells beneath. *a*, Contributing hyphæ; *b*, pseudoparenchyma layer; *c*, sporophores. Compare Pl. 58, A. × 1,050.

C.—Tangential section in the xylem, showing the cut end of a ray and the manner in which a haustorium (*a*) may rise from the hyphæ in the ray and enter the lumen of the tracheid. × 525.

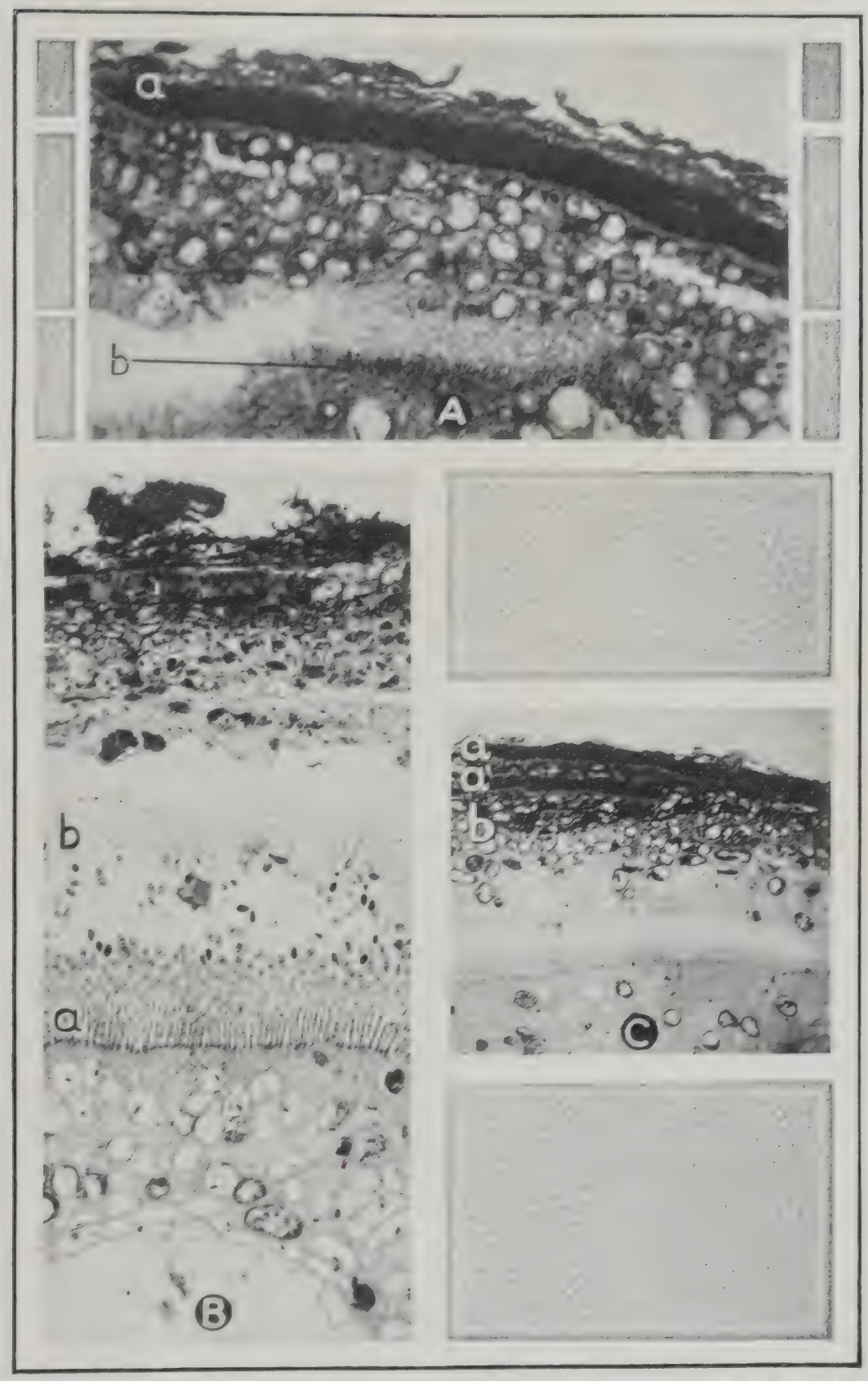
PLATE 50

Cronartium ribicola:

A.—A figure illustrating the relation of the pycnium (*a*), the heavy black line at the top, and young æcium, to the host tissue. The heavily stained cells (*b*) in the region of the young æcium are the fertile cells. Compare Plate 54, B. × 125.

B.—A section through a mature æcium, taken a little to one side of the break in the bark, to show the æciospore chains (*a*), the multilayered peridium (*b*), and the overlying host tissue. × 75.

C.—A similar section showing a double pycnial layer (*a*, a_1), and the location of the cork cambium (*b*) which cuts out the old pycnium. × 40.


a
b
A
b
a
B
a
a
b
C

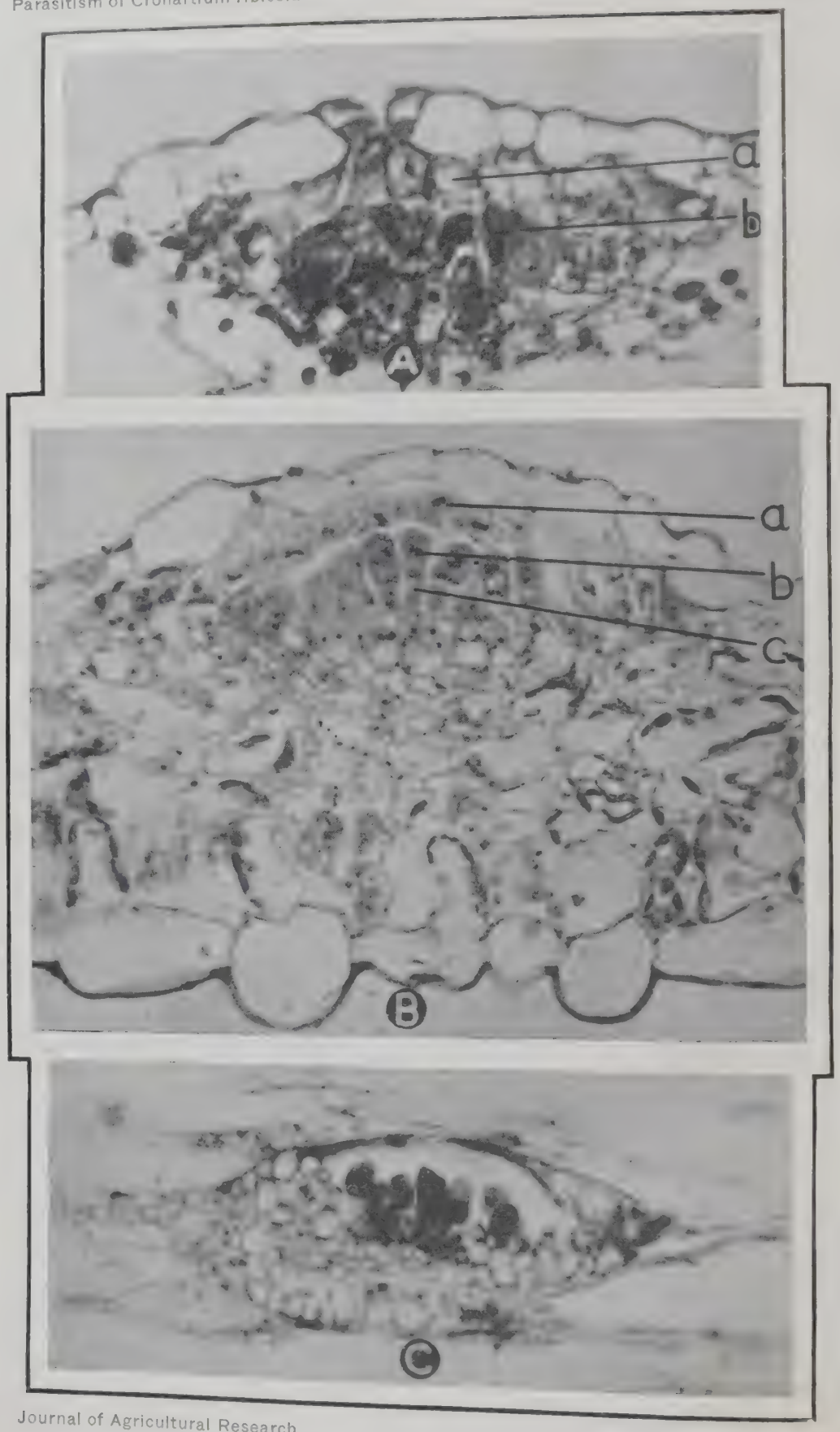

a
b
A
a
b
c
B
C

PLATE 51

Cronartium ribicola:

A.—A median section through a young uredinium forming in the space beneath a stoma. *a*, Peridial cells; *b*, young urediniospores. The photomicrograph illustrates a stage in the development of the uredinium midway between the stages shown in Plate 51, B, and 55, B. × 650.

B.—A section through the same uredinium as that shown in Plate 55, C, taken to one side of the break in the peridium, toward the edge of the sorus. *a*, Peridial cells; *b*, a young urediniospore; *c*, A stalk cell. × 525.

C.—An internal uredinium from the cortex of *Ribes hirtellum*. × 315.

PLATE 52

Cronartium ribicola:

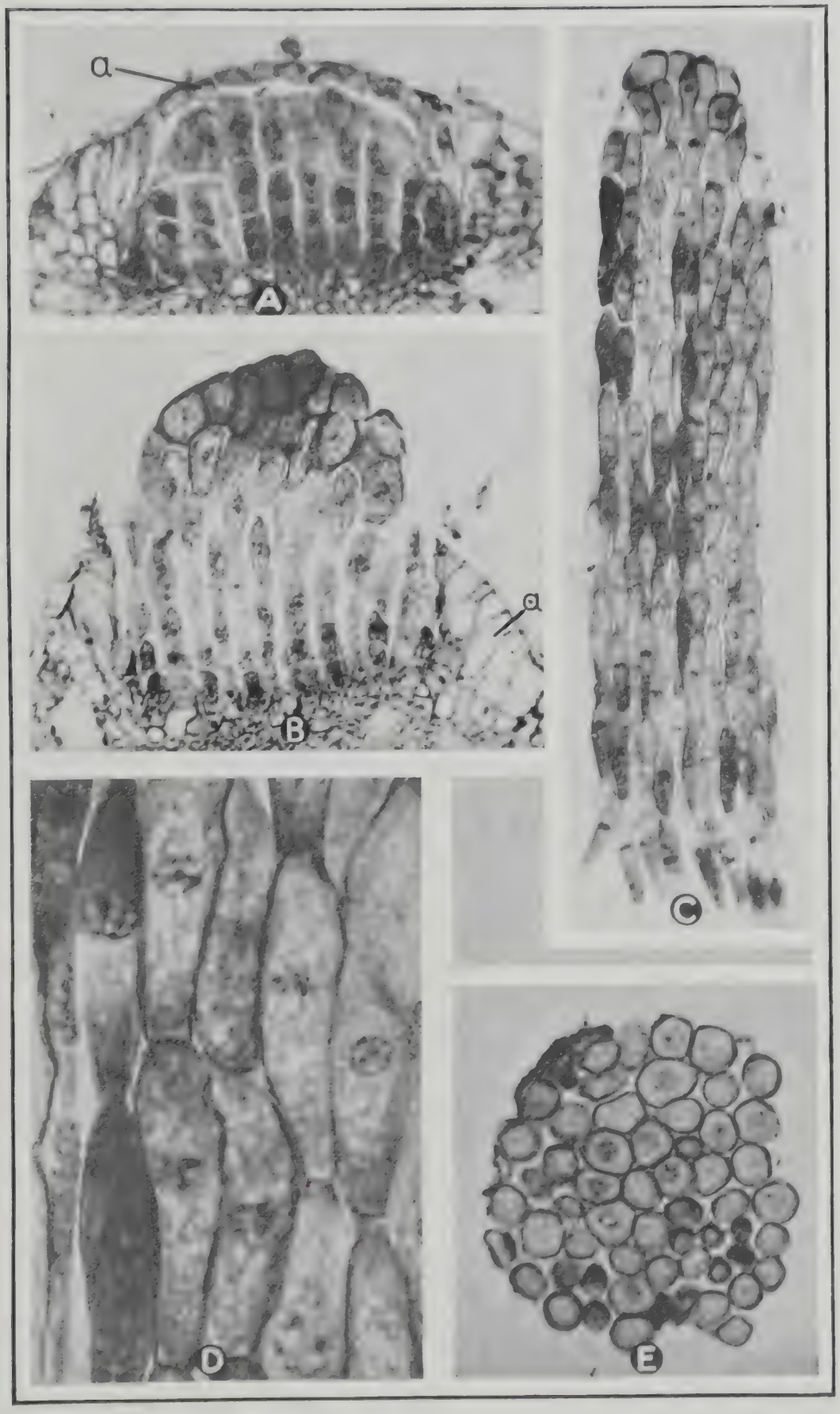
A.—A section of a young telial column. Note the overlying peridial cells (*a*) and the row arrangement of the developing teliospores. The epidermal cells have been torn off. × 355.

B.—A later stage in the development of the telial column. Note the shape of the tip cells and the character of the parenchyma-like cells (*a*) surrounding the base of the young column (cf. Pl. 55, C). The cells near the base of the column are binucleate, while those at the tip are uninucleate. × 355.

C.—A longitudinal section of a mature column. × 250.

D.—Higher power view of the same section, showing the arrangement of the individual spores, and the size of the nuclei. × 1,050.

E.—A cross section of a small mature column. × 525.


a
A
a
B
C
D
E

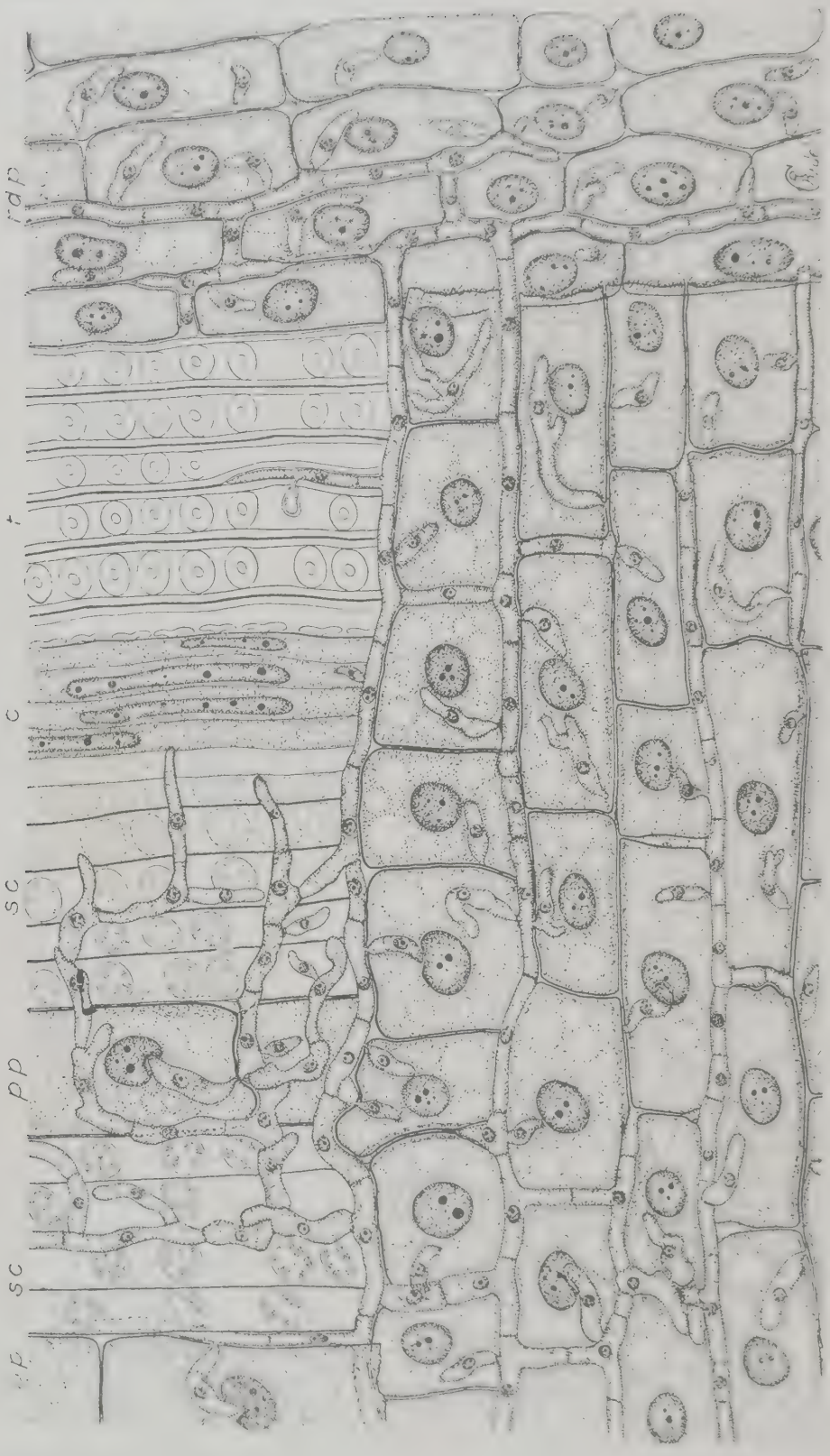

rdp
t
c
sc
pp
sc
ep

PLATE 53

Cronartium ribicola:

A drawing to show the intimate relation of the mycelium of the parasite to the host cells. *pp*, Phloem parenchyma; *sc*, sieve cells; *c*, cambium cells; *t*, tracheids; *rdp*, resin-duct parenchyma. The number of haustoria represented as entering the cells in this drawing is not abnormally large. The drawing was made from two serial sections from the same area by means of a projection apparatus and a camera lucida and has been diagrammatized only so far as was necessary to bring some of the elements to a proper level for drawing. In a few cases nuclei, which were not present in the sections, were supplied for both host and parasite cells. Note the different shapes and sizes of the haustoria and the general character of the hyphæ lying between the cells. × 500.

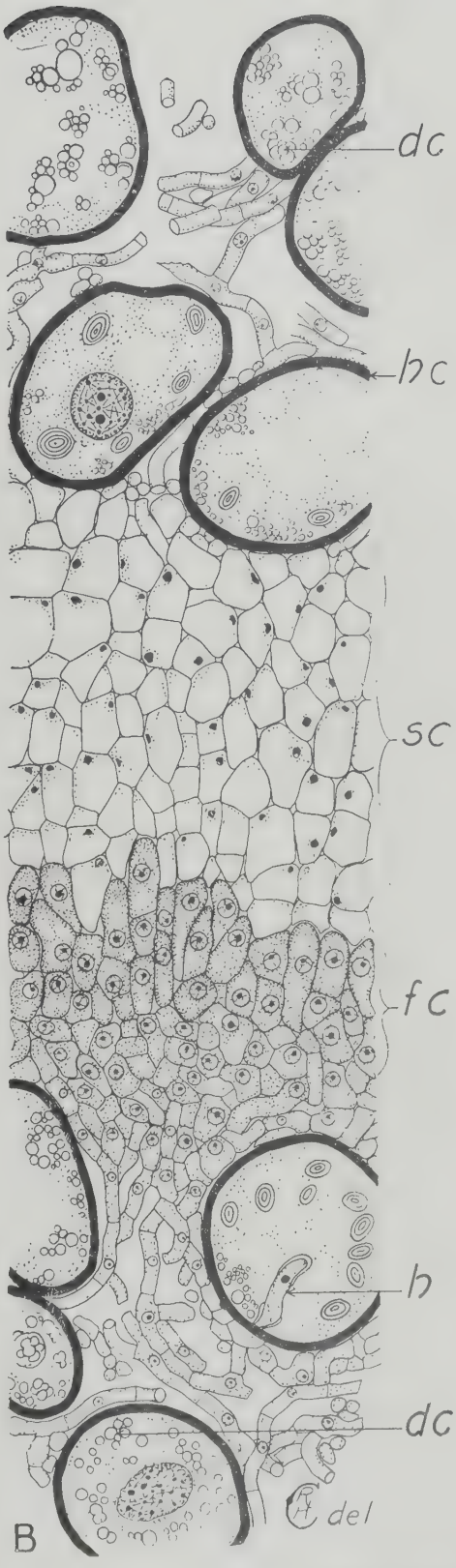
PLATE 54

Cronartium ribicola:

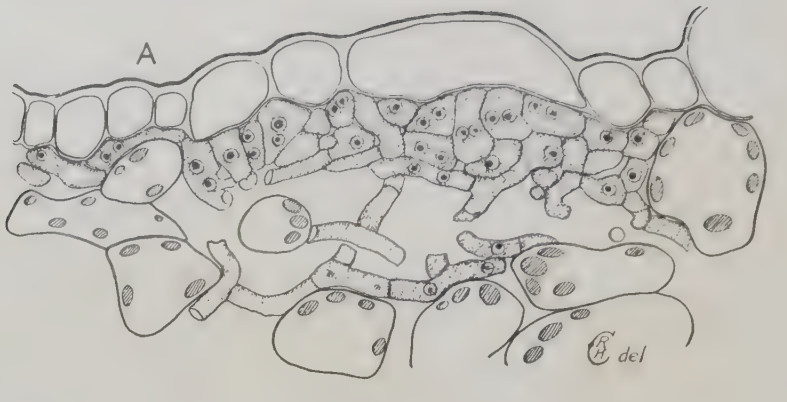
A.—A drawing of an infected 12-year-old main stem. The infection entered the main stem along the small branch, the stub of which is shown at the right of the figure. *a*, The advancing edge of the infection; *b*, the pycnial area. The black dots are the pycnial spots; *c*, the æcial area on which the bark is cracked and broken. In another season the æcial area would spread over the pycnial area (*b*), and the pycnial area would be advanced as far as the boundary (*a*) under normal conditions. The boundary (*a*) would be proportionately advanced also. The specimen from which the drawing was made was collected in August, 1917. × ½.

B.—Drawing of a section through part of a young æcium showing the relation of the fertile cells with their denser protoplasmic contents to the overlying sterile cells, in which the cytoplasm and nuclei have begun to go to pieces. The manner in which the adjacent host cells are forced apart by the fungus cells is also shown. *dc*, decomposition products in the host cells; *hc*, host cell wall; *sc*, sterile cells; *fc*, fertile cells; *h*, haustorium. The elliptical bodies in the host cells represent starch grains. × 400.

a
b
c
A
del


dc
hc
sc
fc
h
dc
B
del

Parasitism of Cronartium ribicola



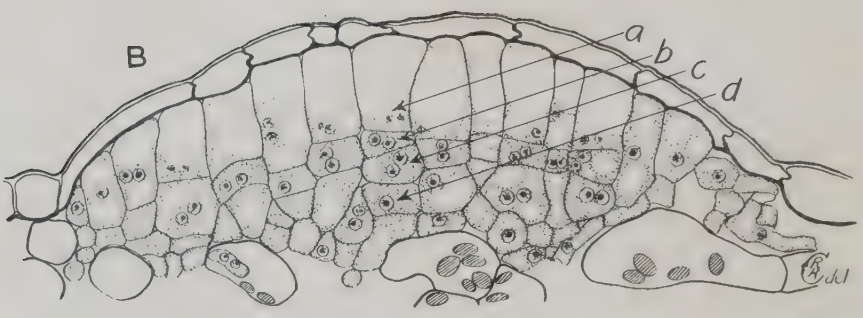


PLATE 55

Cronartium ribicola:

A.—A drawing of a median section through a very young uredinium. Note that the fungus cells are closely compacted against the underside of the host epidermal cells, and that the binucleate cells adjacent to the epidermal cells are oriented with their long axis more or less perpendicular to the epidermis. The section from which the drawing was made was cut just to one side of a stoma. See text for further explanation. × 500.

B.—A drawing of a median section through a young uredinium, somewhat older than that illustrated in figure A. *a*, A peridial cell; *b*, a young urediniospore; *c*, a cell which is homologous to the stalk cell of an older sorus; *d*, a basal cell. See text for further explanation. × 500.

C.—A drawing of a median section through a small mature uredinium. *a*, A binucleate basal cell; *b*, a tetranucleate basal cell just before the urediniospore initial is cut off; *c*, a secondary urediniospore initial which has just been cut off from the basal cell; *d*, the urediniospore initial has divided into a urediniospore and a stalk cell; *e*, a mature urediniospore which is still connected with the basal cell by a collapsed stalk cell; *f*, crushed epidermal cells of the host; *g*, the bank of parenchyma-like cells which encircle the sorus. The cells of this group, which lie next to the epidermis, are homologous to the thickened peridial cells which overlie the greater part of the uredinium. Note the comparatively small opening in the peridium. The drawing has been slightly diagrammatized for the sake of completeness by combining some of the features from two sections. Compare Plate 51, B. × 500.

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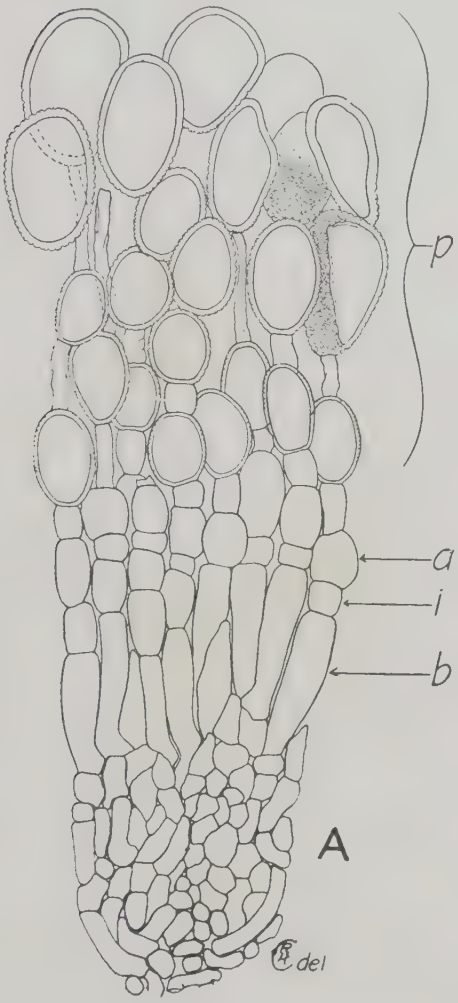
PLATE 56

Cronartium ribicola:

A.—A drawing of the cell relations near the edge of an æcium to illustrate the formation of the multilayered æcial peridium. *a*, A young æciospore; *i*, an intercalary cell; *b*, the basal cell of the chain; *p*, the potential æciospores which are being modified into peridial cells. Note the wall markings and the long degenerating intercalary cells. × 400.

B.—A drawing of a section through a mature peridium, taken from the same series as the photomicrographs in Plate 50, B. × 400.

C.—A drawing of a short mature telial column in which the teliospores (*a*) have germinated, producing promycelia and sporidia (*b*). See Plate 57 for details of the process. ×170. Drawn by Miss Minnie W. Taylor.


p
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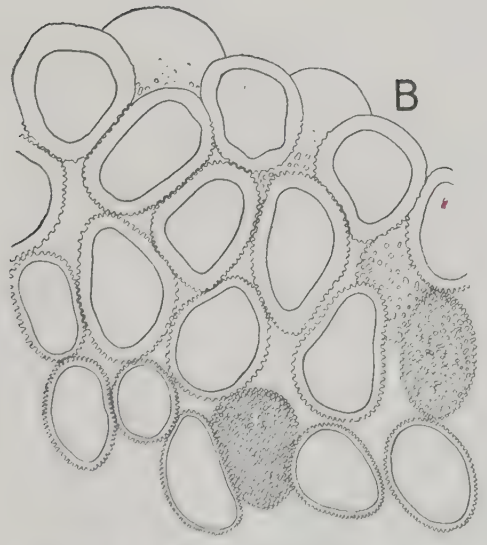
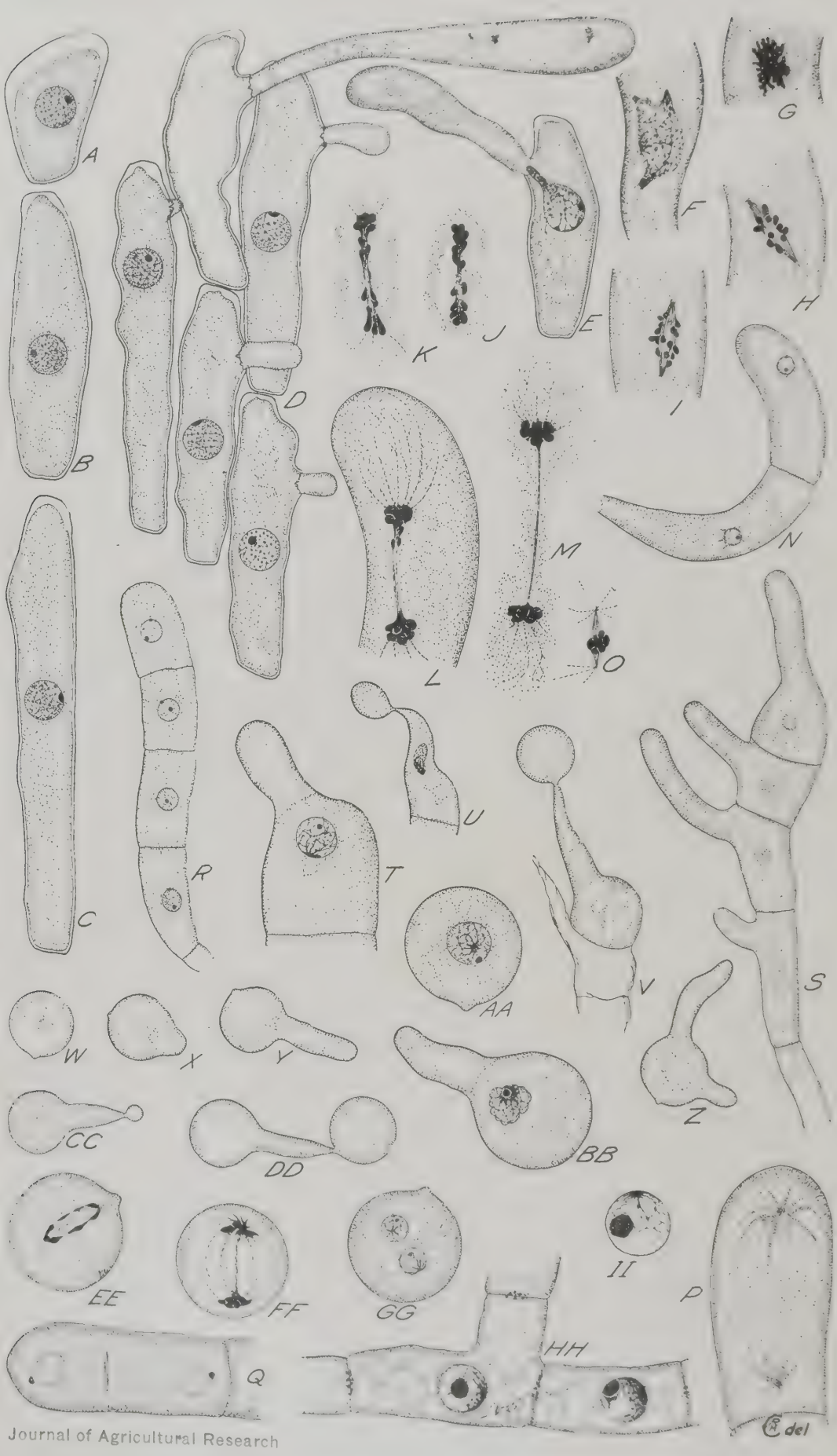
i
b
A
del

b
a
C


B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Z
AA
BB
CC
DD
EE
FF
GG
HH
II

PLATE 57

Cronartium ribicola:

A.—A mature teliospore from the tip of a column. × 850.

B.—A mature teliospore from the side of a column. × 850.

C.—A mature teliospore from the side of a column. × 850.

D.—Five germinating teliosporesfrom a longitudinal section of a column. The nucleus in the promycelium from the upper teliospore is in the telophase of the primary division. × 850.

E.—A germinating teliospore. The nucleus is about to pass through the germ pore into the promycelium. × 850.

F.—Early prophase of the primary division in the promycelium. × 1,700.

G.—Late prophase of the primary division in the promycelium. The chromatin is in the form of complex tangle. ×1,700.

H.—Early anaphase of the primary division in the promycelium. Eleven chromosomes are visible. ×1,700.

I.—Later anaphase than that shown in figure H. Sixteen chromosomes visible. × 1,700.

J, K.—Two anaphase stages of the primary division. The chromosomes are well advanced toward the poles. ×1,700.

L.—Late anaphase of the primary division. The chromosomes are condensing into deeply staining clumps, but the individuals are still discernible in some cases. ×1,700.

M.—End of the anaphase of the primary division. The fibres connecting the two chromatin groups are drawn to a thin fading strand. Note the radiations in the cytoplams in this and the preceding figure. ×1,700.

N.—The 2-celled promycelium. ×850.

O.—Metaphase of the second division. × 1,700.

P.—Telophase of the second division. Peculiar cytoplasmic radiations run from the reorganizing nuclei. × 1,700.

Q.—The reorganizing nuclei after the second division. × 1,700.

R.—The completed promycelium. Each nucleus shows a small nucleolus and a definite centrosome. × 850.

S.—Surface view of a germinating promycelium. × 850.

T.—The tip cell of a germinating promycelium. The nucleus exhibits definite polarization. × 1,700.

U.—A little later stage than the last. The nucleus is passing into the sterigma on its way to the sporidium. × 850.

V.—Surface view of a tip cell of a promycelium bearing a sterigma and a nearly mature sporidium. × 850.

W.—A mature sporidium. The papilla marks the point of attachment of the sterigma. × 850.

X, Y, Z.—Steps in the germination of the sporidia. × 850.

AA.—Sectional view of a mature sporidium. The nucleus shows polarization. × 1,700.

BB.—Sectional view of a germinating sporidium. The nucleus appears to be moving toward the germ tube and preparing to divide. × 1,700.

CC, DD.—Two stages in the formation of secondary sporidia. × 850.

EE.—Midanaphase of the division of the sporidium nucleus. × 1,700.

FF.—Late anaphase of the same. × 1,700.

GG.—Sectional view of a binucleate sporidium. × 1,700.

HH.—Two cells from the vegetative mycelium in the pine. × 1,700.

II.—A definitely polarized nucleus from the vegetative mycelium in the pine, located just beneath the fertile layer of the young æcium. × 1,700.

PLATE 58

Cronartium ribicola:

A.—The elements of the pycnium. The cells at the base are almost empty. Above them are the short branching trunks which bear the sporophores. *a*, A sporophore; *b*, pycniospores in sectional view; *c*, the cytoplasm is constricted just beneath the spore; *d*, the nucleus is dividing. × 1,700.

B.—An active thin-walled haustorium from a pine host cell. × 1,700.

C, D, E.—Old haustoria. C and D have basal cuplike sheaths and tip sheaths. E is completely inclosed in a thick sheath. × 850.

F.—Telophase of division of one of the cells of the fertile layer to form a sterile cell. ×1,700.

G.—A newly formed sterile cell. × 1,700.

H.—A large polarized nucleus from the fertile layer. × 1,700.

I.—An æcial basal cell resulting from the fusion of two adjacent cells of the fertile layer. Compare the size of the nuclei with that of the nucleus shown in Pl. 57, II. × 1,700.

J.—A diagram of a basal cell resulting from the fusion of two cells from different levels. × 850.

K.—A diagram of a trinculeate irregular basal cell from the tip of which a trinucleate æciospore initial has been cut off. × 850.

L.—A diagram of part of an irregular compound fusion cell. × 850.

M.—A basal cell with the nuclei in early prophase. The centrosome in the left nucleus has apparently divided. *b*. An æciospore initial cell with the nuclei in midanaphase. × 1,700.

N.—Part of a basal cell. The nuclei in prophase. × 1,700.

O.—A later stage than the preceding. The spindle and centrosomes are just visible in the upper nucleus. × 1,700

P.—Metaphase of the division in the basal cell. A centrosome is evident at each of the poles of the spindles. The chromatin is condensed into chromosomes. The nucleoli have moved off and begun to fade. × 1,700.

Q.—Early anaphase, a little later than the stage in figure P. × 850.

R.—Midanaphase of the division. The chromosomes are moving toward the poles along the outside of the spindle. × 1,700.

S.—A later stage of anaphase than in figure R. × 1700.

T.—Final anaphase. The chromatin is condensing in two groups at each pole. × 1,700.

U.—Telophase. The two groups for each pole are still distinct. × 1,700.

V.—Telophase. The two groups at each pole have condensed to single masses. The wall which will separate the æciospore initial from the basal is beginning to form at *a*. × 1,700.

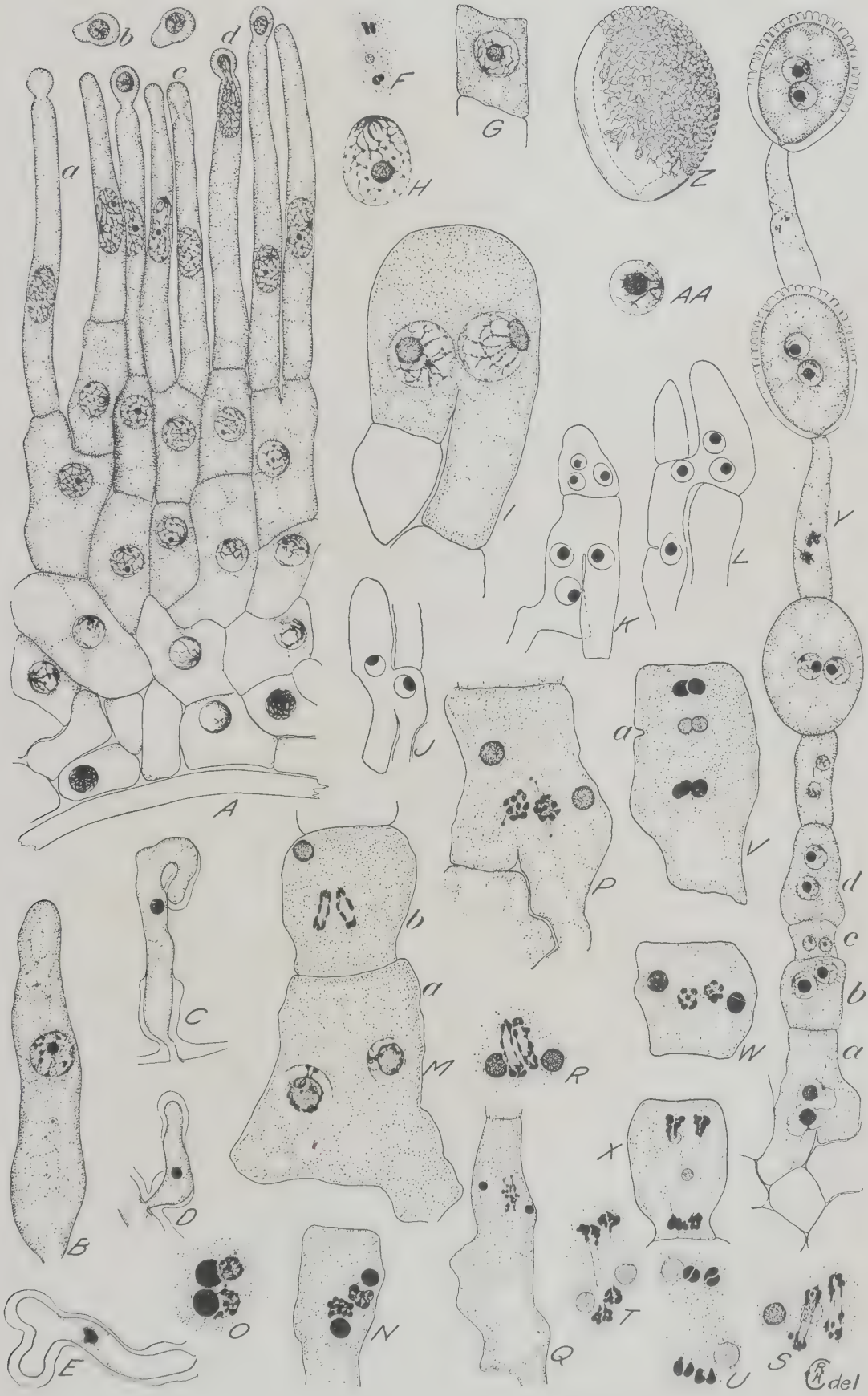
W.—Prophase of the division in the æciospore initial. × 1,700.

X.—Final telophase of the same. × 1,700.

Y.—An æciospore chain in section view. *a*, The basal cell; *b*, an æciospore initial, *c*, an intercalary cell; *d*, a young æciospore. The nuclei in the upper intercalary cells are degenerating. × 850.

Z.—A large mature æciospore in surface view. × 850.

AA.—A nucleus from a mature æciospore. × 1,700.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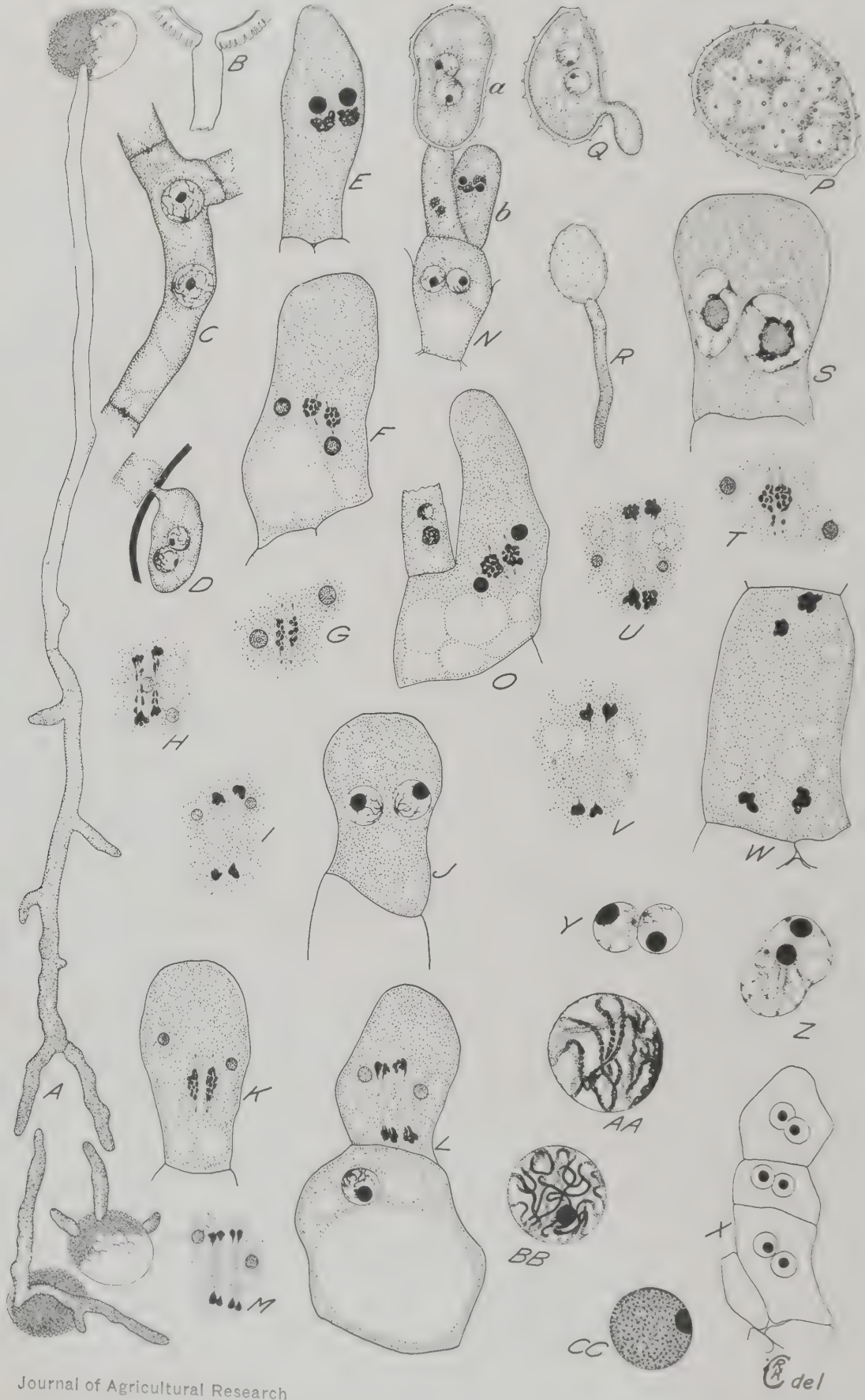
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PLATE 59

Cronartium ribicola:

A.—Germinating æciospores. × 425.

B.—Sectional view of the æciospore wall showing the manner in which the germ tube is constricted. × 850.

C.—A binucleate cell from the mycelium in a leaf of *Ribes* sp. The nuclei are polarized as in the uninucleate mycelium. × 1,700.

D.—A binucleate haustorium from a host cell of *Ribes* sp. × 1,700.

E.—A uredinial basal cell. The nuclei are in prophase. × 1,700.

F.—Metaphase of the primary division in the basal cell. The chromosomes are visible as distinct units. × 1,700.

G.—Early anaphase of the same division. × 1,700.

H.—A later stage of the anaphase. × 1,700.

I.—Final anaphase. The two groups at each pole are not clearly distinct in this figure. × 1,700.

J.—A binucleate urediniospore initial. The nuclei are polarized. × 1,700.

K.—Metaphase or early anaphase of the division in the initial. × 1,700.

L.—Late anaphase in the initial. The chromatin is condensing in two groups at each pole. The second nucleus in the basal cell has been cut away. × 1,700.

M.—Late anaphase group from the initial, for comparison with figure L. × 1,700.

N.—A basal cell bearing a stalk cell surmounted by a nearly mature urediniospore (*a*), and a secondary urediniospore initial (*b*). The nuclei in the latter are in prophase. × 850.

O. Metaphase of the secondary division in the basal cell, preparatory to the formation of a secondary urediniospore initial. × 1,700.

P. A mature urediniospore. × 850.

Q. A germinating urediniospore in sectional view. × 850.

R.—A germinating urediniospore. × 425.

S.—A telial basal cell. Both nuclei show centrosomes and polarization phenomena. × 1,700.

T.—Metaphase of the division in the telial basal cell. × 1,700.

U.—Late anaphase of the same. × 1,700.

V.—Final anaphase of the same. × 1,700.

W.—Telophase of the same. × 1,700.

X.—A diagram of a telial unit column. Two young binucleate teliospores surmount the basal cell. × 850.

Y.—The two nuclei of the young teliospore just previous to fusion. Note the centrosomes. × 1,700.

Z. The two nuclei in the process of fusion. Two nucleoli and two centrosomes are still visible. × 1,700.

AA.—The large fusion nucleus. The chromatin is in heavy strands. × 1,700.

BB.—The fusion nucleus, slightly decreased in size. The chromatin strands are finer than in the preceding stage. × 1,700.

CC.—The mature fusion nucleus. The chromatin is in the form of granules. × 1,700.

FURTHER DATA ON THE SUSCEPTIBILITY OF RUTACEOUS PLANTS TO CITRUS-CANKER[1]

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INTRODUCTION

In the present paper results are given of field observations and inoculations with *Pseudomonas citri* upon plants belonging to genera more or less related to Citrus. These results show a wide range of hosts for Citrus-canker, and the possibility of lesions upon plants other than *Citrus* spp. serving as sources of new infection becomes emphasized in Citrus-canker eradication work.

In the present investigation the findings of Jehle[2] as to the susceptibility of *Chalcas* (*Murraya*) *exotica* Millsp., were corroborated and 23 other species representing 20 genera of the Rutaceae were studied.

It seems inadvisable to present the inoculation data here, since over 2,000 innoculations were made. Table I presents the data in a very much condensed form, and the illustrations show a few of the positive results. To anyone interested the complete inoculation data are available at the Bureau of Plant Industry, Washington, D. C. The inoculations and the controls were dried as herbarium specimens and will also be available for examination.

In making the inoculations an infusion of the organism was painted upon the leaf blade, midrib, petiole, or stem, as the case might be, with a small camel's-hair brush, and then the tissue was punctured through the coating of infusion with a needle. The inoculated twig was maintained in a moist condition by wrapping it in paraffin paper, including

[1] The investigations here outlined have been carried on largely at the Lamao Experiment Station of the Division of Plant Industry of the Philippine Bureau of Agriculture. Thanks are due to Mr. A. M. Burton, former Chief, and to Mr. S. Apostol, present Chief of this Division, as well as to Mr. F. G. Galang, Superintendent of the Lamao station. The Citrus collection of the College of Agriculture at Los Banos was also placed at the use of the writer through the courtesy of Dr. C. F. Baker, Dean of the College.

Many of the plants studied, belonging to genera closely related to Citrus are growing in the experimental plots of Mr. W. T. Swingle, Physiologist in Charge of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, for use in the breeding of canker-resistant Citrus fruits and canker-resistant stocks. Permission to use this material has greatly facilitated the work. Thanks are also due to Mr. Swingle for many helpful suggestions. Many plants related to Citrus grow wild in the Philippine Islands. Prof. E. D. Merrill, Botanist of the Bureau of Science, made helpful suggestions as to such plants and identified many of them. His help has been of the greatest value, and is hereby gratefully acknowledged.

It would have been difficult, if not impossible, to carry on this work without the extensive laboratory facilities of the Bureau of Science so freely made available through the courtesy of Dr. A. J. Cox, Director.

[2] Jehle, R. A. Susceptibility of non-citrus plants to bacterium citri. *In* Phytopathology, v. 7, no. 5, p. 339–344, 3 fig. 1917.

with the twig also a small piece of moistened cotton. Control needle punctures with tap water were made for each host plant, and the moist condition was also maintained with paraffin paper and moist cotton. Unless otherwise noted, the positive results observed here occurred only at needle punctures.

TABLE I.—*Summary of results of inoculations on plants of Rutaceae*

No.	Genus and species.	Inoculation No. for reference.	Result.	Remarks.
	SUBFAMILY CITRATAE.			
	(Not of tribe Citreae.)			
1	*Claucena lansium*.....	1046–1050....	Positive.....	Negative for leaves, but positive on petioles and stems; inoculations slow in maturing.
2	*Chalcas exotica*.....	876–880.....	Weakly positive.	Negative for leaves but positive on petioles and stems. Susceptibility only evidenced by slight swelling not evidenced in controls.
	TRIBE CITREAE.			
	SUBTRIBE Feroninae.			
3	*Feronia limonia*.....	41–50.....	Positive.....	Leaves as well as stems show positive results. Reaction takes place slowly.
4	*Feroniella lucida*.....	1361–1370....	.....do.....	Results obtained only for stem. Positive results obtained very readily.
	SUBTRIBE Aeglinae.			
5	*Aegle marmelos*.....	811–816.....	Negative.....	Inoculation attempts made repeatedly on all parts of plants; all negative; believed to be immune.
6	*Chaetospermum glutinosa*....	61–70; 831...	Positive.....	Results obtained very readily in 5 days. Many naturally occurring infections (Pl. 60).
7	*Balsamocitrus gabonensis*....	1341–1350....	Negative.....	This species is believed to be immune.
	SUBTRIBE Lavanginae.			
8	*Hesperethusa crenulata*.....	1291–1310....	Positive.....	Results obtained quickly and readily for both leaves and stems (Pl. 61, A).
9	*Triphasia trifolia*.....	1071–1808....	Negative.....	Stems and leaves negative.
10	*Paramignya longipeduncu-lata.*	771–790.....	Positive.....	Tissue yellow, with oily appearance around punctures, not raised; no such coloration around control; true both for leaves and stems (Pl. 61, B).
11	*Severinia buxifolia*.....	1381–1390....	Negative.....	Believed to be immune; inoculated repeatedly under same conditions which gave positive results on other genera.
	SUBTRIBE CITRINAE.			
12	*Citropsis schweinfurthii*.....	1371–1380....	Positive.....	Readily positive both leaves and stem.
13	*Atalantia citrioides*.....	1331–1335....	.....do.....	Positive for leaves, causing a watery dark discoloration of tissue but no excrescence. Weakly positive on stem (Pl. 62, A).
14	*Atalantia disticha*.....	936–950.....	Weakly positive.	Stem inoculations slightly swollen, not the case with controls. Leaf inoculations slightly discolored, not the case with controls. Very resistant at least
15	*Eremocitrus glauca*.....	1416–1420....	....do.....	Stem inoculations slowly positive.
16	*Fortunella hindsii*.....	1271–1280....	Positive.....	Quickly positive on both leaves and stem (Pl. 62, B).
17	*Fortunella japonica*.....	1031–1040....	Weakly positive.	Leaf blade inoculations definitely negative; inoculations in midrib weakly positive and inoculations in stems slowly but clearly positive. Believed to be highly resistant.
18	*Microcitrus australis*.....	486–505.....	Positive.....	Quickly positive; cankers definite but much smaller than on *Citrus* spp.
19	*Microcitrus australasica.*	1326–1330....	.....do.....	Stem inoculations show cankers; much smaller than on *Citrus* spp., however.

TABLE I.—*Summary of results of inoculations on plants of Rutaceae*—Continued

No.	Genus and species.	Inoculation No. for reference.	Result.	Remarks.
	RUTACEAE OTHER THAN CITRATAE.			
20	*Toddalia asiatica*.....	1401–1410....	Positive.....	Leaves show no excrescences, but a yellowing of tissue not found in control punctures. Stem inoculations are swollen and the tissue is blackened.
21	*Xanthoxylum rhetsa*.....	631–690.....	Negative.....	Leaves, petioles, and stems negative.
22	*Evodia ridleyei*.....	2113–2127....	Positive.....	Both leaves and stem produce light brown eruptions very definite and similar to those on *Citrus* spp. (Pl. 63). There is also evidence that stomatal infections took place.
23	*Evodia latifolia*.....	291–300.....	.....do.....	Leaves clearly negative but stem tissues show excrescences of a brown color similar to those produced on *Citrus* spp.; lesions verym large.
24	*Melicope triphylla*.....	1526–1540....	.....do.....	Leaves clearly negative; stem tissue shows excrescences of brown color similar to those produced on *Citrus* spp.

Field observations corroborating these inoculation data have been made in many cases. Thus, in South China *Severinia buxifolia* occurs naturally, exposed in a number of instances to infection from Citrus-canker in near-by orchards. In no instances were lesions in any way resembling those of Citrus-canker found. *Aegle marmelos* and *Triphasia trifolia* are cultivated at Lamao in the Philippine Islands, surrounded by nursery rows of Citrus trees heavily infected with canker. In no case have lesions similar to those of canker been found on these hosts.

Chalcas exotica is a commonly-grown ornamental in Manila; nothing at all resembling Citrus-canker has ever been found occurring naturally upon it, although sources of infection are in some cases closely present.

Xanthoxylum rhetsa and *Atalantia disticha* occur naturally at Lamao in places where infection with Citrus-canker would be easily possible. No lesions similar to canker have been found on these plants.

Fortunella japonica occurs in orchards at Lamao and also at Los Banos, Philippine Islands. No naturally-occurring cankers have ever been seen on this host. Wolf [1] reports Citrus-canker on kumquats, but does not mention what species were under observation. Swingle [2] also reports canker on kumquats in Japan.

Fortunella japonica, although susceptible to Citrus-canker under the most optimum conditions, should nevertheless be regarded as highly resistant, closely approaching immunity.

On the other hand, *Fortunella hindsii* occurring naturally in South China has been observed frequently with heavy canker infection. Plants

[1] WOLF, Frederick A. CITRUS-CANKER. *In* Jour. Agr. Research, v. 6, no. 2, p. 70. 1916.

[2] U. S. DEPARTMENT OF AGRICULTURE. CITRUS CANKER IN PHILIPPINES. *In* U. S. Dept. Agr. Dept. Circ., v. 1, no. 1, p. 8. 1915.

of this species were found by Prof. G. W. Groff and the writer near the summits of mountains in Kwangtung Province at an altitude of about 1,500 feet. These mountains are heavily eroded and peculiar in having their sides barren and almost entirely free from growth. Plants of *F. hindsii* at the tops of these mountains were therefore very much isolated from commercial Citrus plantings and other sources of canker infection; nevertheless, in almost all cases the species was heavily infected with Citrus-canker. The writer later found plants of the same species upon Victoria Peak, Hongkong, a mountain of very similar type. It is possible that further facts may show that this plant is an original wild host from which Citrus-canker has spread to cultivated species.

Chaetospermum glutinosa occurs both naturally and cultivated at Lamao, and naturally occurring cankers are abundant upon such plants. The susceptibility of *C. glutinosa* to canker is easily greater than that of the sweet orange (*Citrus sinensis*) in the Philippines.

Glycosmis pentaphylla occurs naturally in Kwangtung Province, China, in places where infection from Citrus-canker would be easily possible. No naturally occurring cankers were observed.

Of the positive results obtained in the foregoing tabulated species, *Pseudomonas citri* has been reisolated from *Claucena lansium, Feronia limonia, Feroniella lucida, Chaetospermum glutinosa, Hesperethusa crenulata, Paramignya longipedunculata, Citropsis schweinfurthii, Atalantia citrioides, Fortunella hindsii, Microcitrus australasica, M. australis, Toddalia asiatica,* and *Evodia ridleyei.* Such isolations have been reinoculated on foliage of *Citrus grandis,* and have given positive results in each case. In those positive results not listed as having the organism reisolated the material was collected and dried in the field, where laboratory facilities were not available.

The statements made above as to immunity, since they are based on lack of infection after inoculation with a dense infusion of the causal organism under the most favorable conditions for infection, are probably more substantial than claims made for absence of infection under natural conditions.

The most noteworthy feature of the inoculations is the susceptibility of such very distant relatives as *Evodia ridleyei, E. latifolia,* and *Melicope triphylla.* In these cases the cankers are by no means weakly produced, but form quickly, with a decided swelling of the tissue, which later erupts as on species of Citrus. On *E. ridleyei* the results were evident in two weeks. *Pseudomonas citri,* therefore, is not closely limited to *Citrus* spp., but has a very wide range of host plants within the family Rutaceae.

Severinia buxifolia, Aegle marmelos, and *Balsamocitrus gabonensis* produce no reaction whatever when inoculated with *Pseudomonas citri.* It is believed that these species may be safely called immune to Citrus-canker; this is especially noteworthy, since they are all close relatives of the genus Citrus.

The evidence is that *Xanthoxylum rhetsa* and *Triphasia trifolia* are immune, while *Chalcas* (*Murraya*) *exotica*, *Atalantia disticha*, and *Fortunella* (*Citrus*) *japonica* show positive results only under the most favorable circumstances for infection.

It is noteworthy that some of the immune and highly resistant species possess thick, coriaceous brittle leaves—for example, *Severinia buxifolia*, *Atalantia disticha*, *Fortunella japonica*, and *Chalcas exotica*. This suggests the possibility that resistance to Citrus-canker may be influenced in some measure by histological or morphological differences.

SUMMARY

(1) Inoculation tests with *Pseudominas citri* upon 24 species representing 20 genera of the family Rutaceae show that 19 of the species are susceptible in greater or less degree. It thus appears that Citrus-canker is not closely limited to the genus Citrus, but has a wide range of hosts among the Rutaceae.

(2) *Severinia buxifolia*, *Aegle marmelos*, and *Balsamocitrus gabonensis*, all close relatives of Citrus, may safely be called immune to Citrus-canker. *Xanthoxylum rhetsa* and *Triphasia trifolia* seem to be immune.

(3) *Chalcas* (*Murraya*) *exotica*, *Atalantia disticha*, and *Fortunella* (*Citrus*) *japonica*, also closely related to the genus Citrus, are strongly resistant to Citrus-canker.

(4) *Claucena lansium*, *Feronia limonia*, *Feroniella lucida*, *Chaetospermum glutinosa*, *Hesperethusa crenulata*, *Paramignya longipedunculata*, *Citropsis schweinfurthii*, *Atlantia citrioides*, *Eremocitrus glauca*, *Fortunella hindsii*, *Microcitrus australis*, *M. australasica*, *Toddalia asiatica*, *Evodia ridleyei*, *E. latifolia*, and *Melicope triphylla*, of different relationships to the genus Citrus, all produce positive results when inoculated with *Pseudomonas citri*, at needle punctures. Of these *Claucena lansium* and *Feronia limonia* develop infection very slowly, the others fairly quickly.

(5) *Chaetospermum glutinosa* shows naturally occurring infections of Citrus-canker and in the Philippines its susceptibility is easily greater than that of the sweet orange (*Citrus sinensis*). *Fortunella hindsii* occurs naturally in South China, very much isolated from sources of Citrus-canker infection. The abundance of cankers found on such trees gives rise to the theory that this species may have been an original wild host from which Citrus-canker spread to cultivated species.

PLATE 60

Naturally occurring Citrus-canker lesions on leaves of *Chaetospermum glutinosa*. Natural size.

B

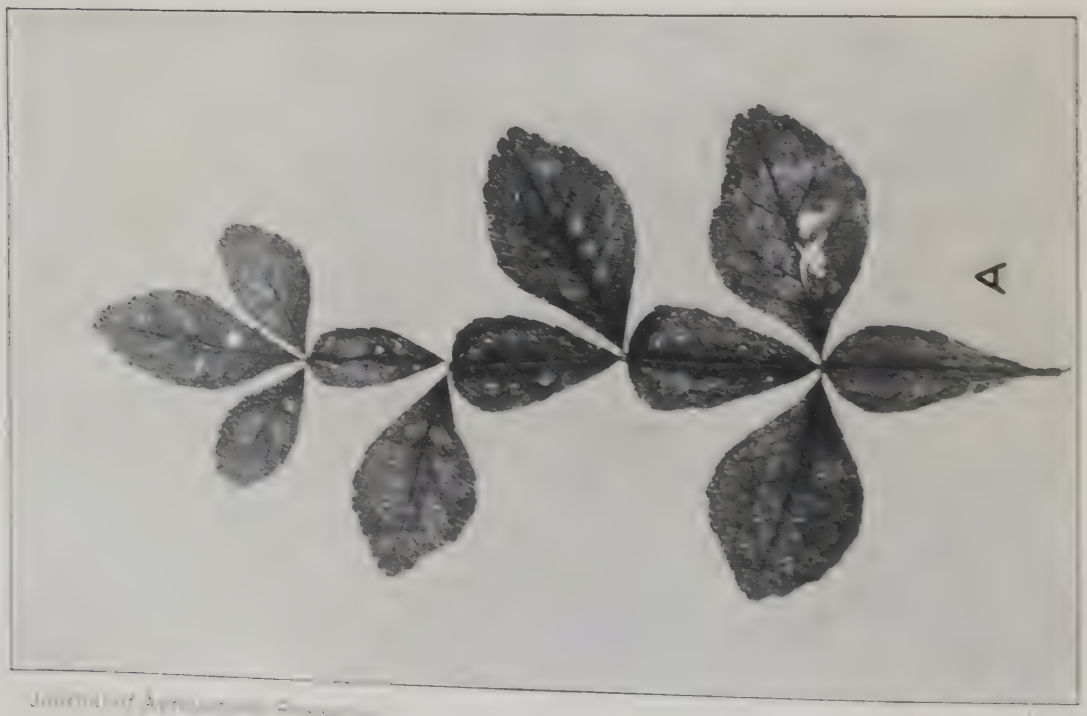

A

PLATE 61

A.—*Hesperethusa crenulata*, showing discolorations resulting from inoculations with *Pseudomonas citri*. Natural size.

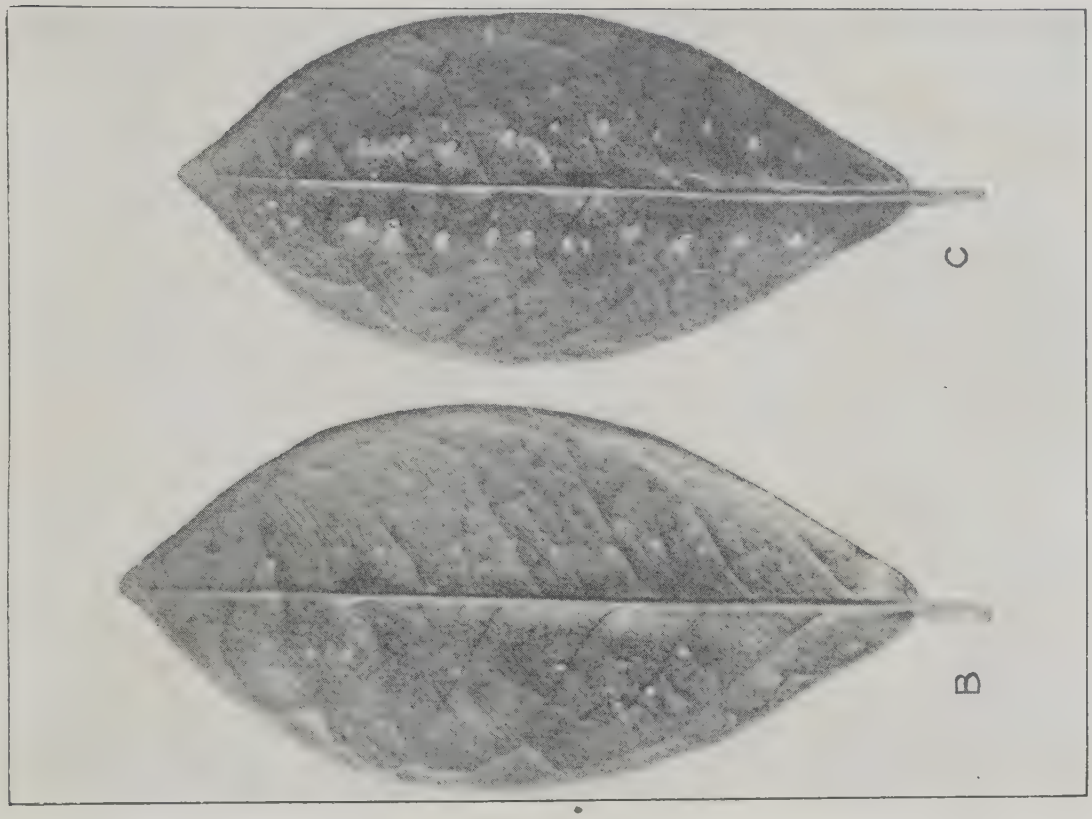
B.—*Paramignya longipedunculata*, showing discolorations around punctures made with *P. citri* on leaves. Such discolorations did not develop at punctures made with river water. Natural size.

PLATE 62

A.—*Atalantia citrioides*, showing positive results following inoculation with *Pseudomonas citri*. Natural size.

B.—*Fortunella hindsii*, showing results of inoculation with rain water on leaves. Natural size.

C.—*Fortunella hindsii*, showing results of inoculation with *P. citri* on leaves. Natural size.


C
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A

A
B
C

PLATE 63

Evodia ridleyei:

A.—Stem inoculated with tap water.
B, C.—Two twigs inoculated with *Pseudomonas citri*. Natural size.

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